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Exercise for Cardiovascular Disease Prevention and Treatment

From Molecular to Clinical, Part 1

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Part I
Overview

Chapter 1
Physical Inactivity and the Economic and Health Burdens Due to Cardiovascular Disease: Exercise as Medicine

Mark Hamer, Gary O'Donovan, and Marie Murphy

Abstract Leisure time physical activity, or exercise, has been described as today's best buy in public health. Physical inactivity is responsible for around 10% of all deaths and physical inactivity costs global healthcare systems billions of dollars each year. Here, we describe the human and economic costs of cardiovascular disease. Then, we explain that physical inactivity is a major modifiable risk factor for cardiovascular disease. The evidence of the role of physical activity in the primary prevention of cardiovascular disease is reviewed and we make the case that exercise is medicine.

Keywords Exercise • Cardiovascular disease • Prevalence • Medicine

1 Introduction

Cardiovascular disease (CVD) is a term used to describe a range of conditions affecting the heart and the vessels that carry blood around the body. These conditions include abnormalities in the structure or function of the heart (heart failure, rheumatic heart disease and cardiomyopathy) or the blood vessels supplying the heart (coronary or ischaemic heart disease), the brain (cerebrovascular disease or stroke), or the peripheral vascular system (including hypertension, claudication and thrombosis). CVD is the main cause of death in Europe, accounting for 45% of all deaths [1]. Half of all CVD deaths are caused by coronary heart disease (CHD), while a further third are directly attributable to stroke. Most heart attacks and many

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strokes occur as a result of atherosclerosis (narrowing of the arteries) and thrombosis (blood clotting). The narrowing of the arteries in atherosclerosis is an inflammatory process characterised by the accumulation of low-density lipoprotein cholesterol (LDL-C) in the artery wall and the formation of atherosclerotic plaques. Angina (pain in the chest) can occur when an atherosclerotic plaque become large enough to compromise blood flow in a coronary artery. However, many heart attacks occur without warning when a plaque ruptures, its thrombotic content is exposed to blood, and a large clot is formed [2]. In fact, autopsy data suggest that around 90% of heart attacks occur when a blood clot is superimposed on an already narrow artery. While gender, age and genetics play roles in CVD, it is now well established that environmental factors such as smoking and lack of physical activity are the major determinants of disease risk in most individuals in developed nations. In this chapter we describe the human and economic burdens of CVD in the EU and we outline the major risk factors for this group of diseases. The evidence on the role of physical activity in the primary prevention of CVD will be reviewed with a view to making a case that *exercise is medicine*.

2 The Burden of Cardiovascular Disease

2.1 Human Costs

Cardiovascular disease is responsible for four million deaths across Europe. Coronary heart disease is the single most common cause of death, accounting for approximately 20% of all deaths, while stroke and other CVD are responsible for 11% and 14% of all deaths, respectively [1]. The human costs of caring for individuals with cardiovascular disease has also been calculated. It is estimated that over half a million people in the UK alone provide over 500 million hours of informal care for family and friends with CVD [3]. In addition to being a major cause of death, CVD significantly reduces quality of life for many individuals in the UK. Disability Adjusted Life Years is a measure of the years of life lost due to premature death and the years of healthy life lost through disability. It is estimated that more than 80 million fully healthy life years are lost to CVD every year in the European Region [4]. The human costs of physical inactivity are described in Sect. 3.2.

2.2 Economic Costs

Cardiovascular disease is estimated to cost the EU economy almost €196 billion a year. Some 54% of the total cost is due to direct health care costs, 24% to productivity losses, and 22% to the informal care of people with CVD. The cost to the health care systems of over €106 billion represents a cost per capita of €212 per annum [5]. The economic costs of physical inactivity are described in Sect. 3.2.

3 Risk Factors for Cardiovascular Disease

Over 300 risk factors have been identified for CVD. In order to be classified as a major risk factor, however, an exposure or behaviour must meet three criteria: (1) an independent association with CVD; (2) a high prevalence in many populations; and (3) its treatment and control can result in reduced risk. The contribution of individual risk factors may vary depending on the socioeconomic status of the country and the prevailing forms of CVD. In Britain, around 80% of CHD events in middle-aged men are thought to be explained by total cholesterol, blood pressure and cigarette smoking [6]. High blood pressure presents a greater risk for ischaemic stroke, while high cholesterol is a stronger predictor of CHD risk. Herein, we discuss 'traditional' and 'novel' risk factors because 20–50% of CHD events are not explained by traditional risk factors [6].

3.1 Non-modifiable CVD Risk Factors

Risk factors that cannot be modified include age, male gender, and a family history of premature CVD. Advancing age is one of the most powerful risk factors for CVD; for example, the risk of stroke doubles every decade past the age of 55 years. Cardiovascular disease is not inevitable, but age is a surrogate measure of exposure to all other risk factors. Historically, men experience a higher rate of CVD than women at a younger age, and prior to menopause women have better cardiovascular risk profiles than men [7]. For example, pre-menopausal women demonstrate lower levels of blood pressure and LDL-C and higher levels of high-density lipoprotein cholesterol (LDL-C). Given that risk profiles of women and men become similar following the menopause, the cardio-protective effects of female sex hormones may partly explain the gender difference in CVD risk. Postmenopausal women appear to be at a distinct disadvantage because diabetes carries a significantly greater risk of CVD and systolic hypertension becomes more frequent in older women.

3.2 Physical Inactivity and Other Modifiable CVD Risk Factors

Some of the major CVD risk factors are modifiable, in that they can be prevented, treated and controlled. The World Health Organisation highlights seven major modifiable CVD risk factors, which include raised blood pressure, abnormal blood lipids, tobacco use, physical inactivity, obesity, unhealthy diet, and diabetes mellitus. Physical inactivity has been defined as an activity level insufficient to meet the World Health Organisation recommendation of at least 150 min per week of moderate-intensity aerobic activity, or at least 75 min per week of vigorous-intensity

aerobic activity, or equivalent combinations [8]. It has been estimated that, if physical inactivity were to decrease by 25%, more than a million deaths worldwide would be avoided each year (the population attributable fraction for all-cause mortality is 9%, ranging from 4% in low-income countries to 11% in high-income countries) [8]. Reductions in smoking, blood pressure, and cholesterol have explained approximately 50% of the decline in CVD events over the last 25 years in Britain [9]. The relative risk of heart disease per unit change in cholesterol has been shown to decrease with age and blood pressure, although the positive association persists into older age [10]. Pharmacological therapies that have been employed to control elevated cholesterol and blood pressure demonstrate a favourable effect on cardiovascular outcomes. For example, statin therapy was associated with a 12% proportional reduction in all-cause mortality per $mmol \cdot l^{-1}$ reduction in LDL-C and a 19% reduction in the 5-year incidence of coronary events [11]. The recent implementation of comprehensive smoking bans in public places has provided an opportunity to examine the large-scale impact of exposure to tobacco smoke using a natural experiment design. Hospital admission rates for acute myocardial infarction were reduced by 8% as a result of a comprehensive smoking ban in New York State, which was estimated to result in direct healthcare savings of US$56 million per year [12]. While much as been done to tackle smoking, little has been done to tackle physical inactivity [13, 14]. Physical inactivity costs global healthcare systems at least INT$54 billion per year [15] and policy makers have been urged to take physical inactivity more seriously [16].

3.3 Novel Modifiable CVD Risk Factors

A variety of novel circulating biomarkers that reflect inflammation, coagulation, impaired fibrinolysis, and increased blood viscosity have been identified as potential CVD risk factors [17]. There is intense inflammatory activity in atherosclerosis, for example, and inflammatory markers such as interleukin (IL)-6 and C-reactive protein (CRP) may directly influence plaque vulnerability and rupture [18]. However, the clinical utility and causal role of novel risk markers remains widely debated. In several meta-analyses of large scale prospective cohort studies, CRP and fibrinogen were found to be moderately associated with risk of CHD and other cardiovascular outcomes after adjustment for traditional risk factors [19, 20]. There are limited data on the predictive value of novel biomarkers beyond that of traditional risk factors, and existing evidence is equivocal. For example, in the 17-year follow up of 1592 participants from the Edinburgh Artery Study, a wide range of novel biomarkers provided very little prognostic information for incident CVD over and above traditional risk factors [21]. In contrast, prospective data from the Women's Health Study suggested that fibrinogen and CRP provided additive value to traditional risk factors in predicting incident CVD [22].

Geneticists have examined if alleles associated with higher CRP increase risk of CVD because the measurement of plasma CRP at a single point in time may not

adequately reflect an individual's cumulative inflammatory burden. Transgenic studies of over-expression of human CRP have demonstrated no influence on the development of atherosclerosis in mice. In prospective studies in humans the association between polymorphisms of the CRP gene and incident CVD have produced conflicting findings. For example, although CRP genotype was strongly associated with plasma CRP concentration, there was little association between CRP genotype and risk of CVD events in the Physician's Heath Study, the Framingham Heart Study, or the Rotterdam Study. This contrasts with the Cardiovascular Health Study where a strong independent association between CRP genotype and fatal CVD events was observed [23]. Confirmation of these findings in other large population studies of older adults with large numbers of fatal events will be important for clarifying the role of the CRP gene and risk of CVD. Further research is also required to determine the clinical utility of apolipoproteins, adiponectin and other biomarkers because the existing evidence is largely equivocal [24, 25].

3.4 Risk Factor Clustering

The presence of two or more risk factors dramatically increases CVD risk. For example, there are 43 CVD deaths per 10,000 person-years in middle-aged men who smoke, have high blood pressure, and high cholesterol compared to three CVD deaths per 10,000 person-years in middle-aged men without these risk factors [26]. Accordingly, modern treatment regimes have focussed on risk factor clustering to identify those individuals at greatest risk.

4 Physical Activity and Cardiovascular Disease: The Evidence

4.1 Population Based Studies

Prospective cohort designs allow groups of individuals with differing levels of physical activity to be followed for a period of time to determine the relationship between physical activity and CVD risk. Since the early work of Jerry Morris and Ralph Paffenbarger, the role of regular physical activity in the prevention of CVD has been well established. In the 1950s, Morris and colleagues [27] demonstrated that the CHD death rates of bus conductors and postmen were half those of comparably inactive bus drivers and telephonists. Since then, the assessment of physical activity exposure has become more refined with the use of validated self-reported physical activity questionnaires. In the Harvard Alumni Health Study, for example, Paffenbarger and colleagues [28] questioned 16,936 alumni about the daily number of blocks walked and flights of stairs climbed, and about the frequency and duration

of sporting and recreational activities. Regular stair climbing and strenuous sports play were associated with reduced risk of CHD during 6–10 years of follow-up, whilst student athleticism offered no protection. More recently, the Health Professionals' Follow-Up Study, that consisted of 44,452 health professionals followed between 1986 and 1998, is noteworthy because of its large sample size and rigorous methodology [29]. In this study, various forms of activity conferred protection against CHD, including regular walking (18% risk reduction) and 1 h of running per week (42% risk reduction). Men who increased their exercise intensity from low to vigorous over time had a CHD risk reduction of 12%. There has been considerable recent interest in the relationships between physical activity, sedentary behavior and health. The culmination of this research was Ekelund and colleagues' [30] in a review of 16 studies which included more than one million adults. They found that sedentary behavior was associated with all-cause mortality in the entire sample. Importantly, they also found that sedentary behavior was not associated with all-cause mortality in those who were "highly active" (that is, those who reported about 60–75 min per day of moderate activity) suggesting that high levels of activity may offset the negative effects of sedentariness.

Although there is now irrefutable epidemiological evidence that regular physical activity plays an important role in the prevention of CVD, this evidence has been largely derived from studies of white males. More recent studies have therefore attempted to examine associations between physical activity and CVD risk in women and non-white populations [31, 32]. The major epidemiological studies on physical activity and CVD among women have emerged from large American cohorts such as the Nurse's Health Study [33], Women's Health [34], and Women's Health Initiative studies [32]. Across studies there appears to be a fairly consistent inverse dose-response association between physical activity and CVD in women, with minimal protection achieved at a level of at least 1 h per week of moderate intensity exercise such as walking [35].

The association between vigorous-intensity activity and mortality may be stronger than the association between moderate-intensity activity and mortality, at least in men [36–40]. Taken together, the epidemiological evidence supports the notion that exercise should be at least moderate intensity and, for many men and women, brisk walking offers protection from CVD [41]. Our understanding of the optimal frequency, intensity, duration and type of activity for CVD risk reduction [42, 43] is still evolving. Several recent analyses of large scale population data have been useful in trying to determine minimal and optimal physical activity dosage. For example, 15 min a day or 90 min a week of moderate-intensity exercise was shown to lower mortality risk in a sample of more than 400,000 adults from Taiwan [44]. Data from a recent meta-analysis of nine cohort studies revealed that undertaking some moderate to vigorous physical activity but less than the guidelines was associated with 22% reduction in mortality risk [45]. In an analysis of over 600,000 adults from the US and Europe, an upper threshold for longevity occurred at 3 to 5 times the physical activity recommendation although the additional benefit over and

above the guideline was modest, leading Arem and colleagues [46] to describe an 'L-shaped' association. We have used data from the Scottish Health Survey (SHS) and the Health Survey for England (HSE) to examine the relationships between physical activity and health. We investigated associations between physical activity patterns and mortality in more than 60,000 participants in SHS and HSE [47]. Leisure-time physical activity was assessed and participants were defined as inactive (reporting no moderate-intensity or vigorous-intensity activities), insufficiently active (reporting less than 150 min per week of moderate-intensity and less than 75 min per week of vigorous-intensity activities), weekend warrior (reporting at least 150 min per week of moderate-intensity or at least 75 min per week of vigorous-intensity activities from one or two sessions), and regularly active (reporting at least 150 min per week of moderate-intensity or at least 75 min per week of vigorous-intensity activities from three or more sessions). All-cause mortality risk was approximately 30% lower and CVD mortality risk was approximately 40% lower in active versus inactive participants; active included the weekend warriors who performed all their exercise in one or two sessions per week. The weekend warriors took part in a relatively high proportion of vigorous-intensity activity and we concluded that quality might be more important than quantity. Vigorous-intensity activity increases cardiorespiratory fitness more than moderate-intensity activity and cardiorespiratory fitness may be a stronger predictor of mortality than smoking, high cholesterol, high blood pressure and other established risk factors [48].

Few epidemiological studies have been designed to examine the mechanisms that mediate the cardio-protective effects of physical activity. In a study of 27,000 apparently healthy women followed for 11 years, most of the reduced risk of CVD associated with being physically active was explained by risk factors measured by the investigators, including inflammatory/haemostatic biomarkers (which explained 33% of the reduced risk), blood pressure (27%), traditional lipids (19%), adiposity (10%), and glycaemic control (9%) [22]. More observational cohort studies and exercise interventions are required to determine whether novel biomarkers explain the 'protective effect' of physical activity in men.

4.2 Exercise Interventions

Although the large population studies have been invaluable in establishing associations between physical activity and CVD, observational studies are prone to bias (that is, the inferential error associated with any process that causes results to vary systematically from the truth). Randomised controlled trials (RCTs) can provide important information on the effect of exercise frequency, exercise intensity, and exercise duration on various CVD risk factors. The findings from some of the larger studies, meta-analyses and systematic reviews on modifiable CVD risk factors are described below.

4.2.1 Blood Pressure

In a meta-analysis of RCTs published up to February 2012 (105 aerobic, 29 dynamic resistance, 14 combined, and 5 isometric training groups), systolic blood pressure was reduced with aerobic, dynamic resistance, and isometric resistance training, but not combined training [49]. Diastolic blood pressure was reduced with aerobic, dynamic resistance, isometric resistance, and combined training. The authors tentatively concluded that isometric handgrip and isometric leg training might result in larger reductions in systolic and diastolic blood pressure than the other modes of training, despite limited evidence currently available.

Although resting blood pressure is a widely used clinical measure of cardiovascular risk, it is well established that exercise causes a reduction in blood pressure during the period immediately following a bout of exercise. Post-exercise hypotension (PEH), or the reduction in blood pressure following a bout of exercise, may last up to 18 hours and may play an important role in the anti-hypertensive effects of exercise. The optimal dose of exercise is not clear, however, Pescatello and colleagues [50] demonstrated that a light intensity (40% maximal oxygen consumption [VO_2 max]) was as effective as a higher intensity bout of exercise (60% VO_2 max) in eliciting significant PEH over the course of a nine-hour ambulatory monitoring period. In contrast, Quinn [51] demonstrated more substantial and sustained PEH over a 24 h period after a bout of higher intensity exercise (75% VO_2 max) compared with lower intensity exercise (50% VO_2 max). There is also some evidence that short bouts of moderate exercise produce more sustained PEH compared to continuous sessions of the same total duration in prehypertensive adults [52].

4.2.2 Lipid Metabolism

Although observational studies suggest that regular activity is associated with favourable lipid profiles, exercise interventions have yielded less consistent results. In randomised, controlled trials of physical activity, the most commonly noted change in blood lipid profiles is an increase in HDL-C concentration with less consistent alterations in triglycerides (TG) and LDL-C concentrations. When HDL-C, TG and LDL-C changes were noted, these were associated with interventions involving weekly energy expenditure in excess of 900–1200 kcal but appear to be independent of the exercise intensity used to achieve this energy expenditure [53, 54]. In a notable study that contained 492 sedentary adults, higher intensity (65–70% of heart rate reserve) or more frequent walking (5–7 days per week) produced more favourable changes in lipid profiles than moderate intensity (45–55% of heart rate reserve) or low frequency (3–4 days per week) walking at 6 months, although the effects were not sustained at 24 months [55]. Alterations in TG concentrations appear to be more likely in men than in women and more likely to accompany weight loss. There is also some evidence that less favourable lipid profiles at baseline are more likely to alter in response to exercise [56]. In a meta-analysis of 13

exercise interventions with overweight and obese adults, Kelley and colleagues [57] noted a significant decrease in fasting triglyceride levels (11%) but no significant changes in other lipoprotein parameters associated with cardiovascular risk. Genetics may also play an important role. For example, in 35 pairs of monozygotic twins who were discordant for vigorous activity, there were significant correlations between the twin pairs for HDL-C and HDL subclasses, thus suggesting a substantial influence of genetics over and above exercise [58]. Data from the Heritage Family Study also suggest that genetic variation largely explains the substantial variability in HDL cholesterol responses to endurance training [59, 60].

In many studies the favourable alterations in blood lipids have been demonstrated exclusively in a fasted state. Given that humans spend the majority of their lives in a post-absorptive state and impaired metabolism of postprandial lipoproteins are implicated in the development of CVD, the role of physical activity in altering postprandial concentrations of blood lipids may be more clinically important [61]. Cross-sectional studies show improved clearance of postprandial triglycerides among regular exercisers compared to the inactive, although these alterations may be due in greater part to the acute effects of a recent exercise bout rather than any chronic adaptation to regular activity [62]. These findings underscore the importance of regular activity and support the recommendation that physical activity bouts should be daily.

4.2.3 Cardiorespiratory Fitness

Steven Blair's seminal work has demonstrated that low cardiorespiratory fitness is among the most powerful predictors of cardiovascular and all-cause mortality in Caucasian men [63]. More recent work has found that low cardiorespiratory fitness predicts mortality in other populations, including women [64] and black men [65]. The addition of cardiorespiratory fitness to traditional risk factors significantly improves the classification of risk and it has been argued that efforts to improve cardiorespiratory fitness should become a standard part of clinical encounters [48].

Fitness is developed by moderate to vigorous intensity activity and cardiorespiratory fitness is therefore an objective measure this type of activity [66]. Indeed, Blair and colleagues have found that cardiovascular mortality is halved in men who become active and fit compared to those who remain inactive and unfit. Progressive manipulation of the training variables or 'dose' (exercise frequency, exercise intensity and exercise duration) is likely to improve cardiorespiratory fitness [67]. Indeed, in a large RCT including 464 overweight post-menopausal women various doses of walking were shown to produce significant gains in fitness [68]. Modest gains in fitness were even observed at the lowest exercise dose of approximately 72 min per week of moderate-intensity activity. The effect of exercise on fitness was similar across age, weight, baseline fitness, and hormone therapy usage, suggesting that the benefits are comparable across a variety of individuals.

4.2.4 Novel Risk Factors

Several studies have considered the role of physical activity in altering haemostatic dysfunction [69]. There is some evidence that a single bout of vigorous (but not moderate) intensity exercise induces a prothrombic state characterised by platelet hyper-aggregability, blood hyper-coagulability and hypo-fibrinolysis [70]. This response to strenuous exercise appears to be more pronounced in individuals who are normally sedentary [71] and may be one of the causes for the transient increase in the risk of cardiac arrest during and after strenuous exercise [72]. These findings support the notion that low- or moderate-intensity exercise is the most suitable starting point for inactive individuals.

Epidemiological evidence suggests a positive relationship between regular exercise and blood haemostasis and inflammatory markers. Fibrinogen, an acute phase reactant protein that plays an important role in blood clotting, is lower in those participating in vigorous physical training even when age, smoking, alcohol intake and body mass are taken into account [73]. Those who take part in regular low- to moderate-intensity physical activity may also demonstrate lower platelet aggregation and adhesion [74]. In a 20-year follow-up to the British Regional Heart Study, Wannamathee and colleagues [75] showed a reduction in coagulation (measured by plasma fibrinogen, plasma and blood viscosity and coagulation factors VIII and IX), inflammatory markers (CRP, white cell count), and increased fibrinolysis (measured by fibrin D-dimer and tissue plasminogen activator antigen) in those who were physically active. These changes were dependent upon current activity level with those who took up at least light activity approaching those who were always active and those who became inactive over the 20 year period demonstrating levels similar to the inactive [75].

Such epidemiological observations are less well-substantiated by exercise interventions. Wang and colleagues have shown that an eight-week programme of moderate-intensity exercise decreases adhesive and aggregative properties of platelets in healthy young men [76] and women [77] and have demonstrated that this beneficial effect is reversed by a similar period of de-conditioning. These authors have also demonstrated that regular exercise training may desensitise the exaggerated platelet reactivity response associated with strenuous exercise in sedentary individuals [78]. Exercise interventions comparing blood fibrinolysis before and after training have yielded equally inconsistent results [79].

Exercise interventions generally appear to demonstrate reductions in inflammatory markers if accompanied by weight loss, especially among women. For example, in 152 female smokers, there were no changes in CRP or fibrinogen after a 12-week exercise program that improved physical fitness, but was not accompanied by significant weight loss [80]. In contrast, a two-year program designed to improve diet and increase physical activity resulted in significant weight loss and reductions in CRP and IL-6 among 120 obese women [81]. However, in another large RCT containing 193 sedentary, mildly obese, dyslipidemic men and women, 6 months' exercise training did not alter levels of CRP despite improvements in fitness, visceral adiposity and subcutaneous adiposity [82]. The equivocal nature of these findings

might best be explained by disparity in the exercise interventions and insufficient training periods, poor adherence levels, differences between characteristics of participants and genetic influences. Indeed, evidence from the Heritage Family study suggests that exercise training only has significant anti-inflammatory effects in sedentary adults with initially elevated CRP levels [83]. Thus, further studies are required to resolve these issues.

4.3 Biological Mechanisms

Given the range of factors that are altered by exercise it seems likely that multiple biological mechanisms are responsible for alterations in CVD risk. Some of these mechanisms are well-understood and supported by empirical evidence, and others are biologically plausible but unsubstantiated. The reductions in blood pressure associated with regular exercise are likely to be due to a combination of nervous system adaptations and vascular adaptations [84], including decreased activation of sympathetic nervous system and increased vasodilation which decreases peripheral resistance. Various mechanisms have been associated with these vascular adaptations [85], for example, exercise induced enhancement of the synthesis and release of nitric oxide and endothelin-1. The favourable alterations in blood lipids as a consequence of exercise are likely to be due an alteration in the activity of key enzymes involved in lipoprotein metabolism. For example, increased lipoprotein lipase activity and decreased hepatic TG lipase activity have been noted after a single bout of exercise [86]. In addition, reductions in cholesterol ester transfer protein concentrations have been reported [87], which might allow slowed catabolism of HDL particles with endurance training. Increases in cardiorespiratory fitness with exercise result from a combination of improvements in cardiac function, oxygen transport, muscle perfusion and alterations in the activity of key enzymes involved in aerobic metabolism. Numerous mechanisms have been connected with the anti-inflammatory effects of exercise. One key mechanism might be related to nuclear factor-κB activation, given that this is a redox sensitive and oxidant-activated transcription factor that regulates inflammation related gene expression. Heat shock proteins that are released during exercise may be a viable mechanism that could explain the training induced changes in toll like receptor 4, which is thought to play an important role in inflammatory pathways [88]. Changes in leukocyte telomere dynamics, which progressively change with age, may also be involved although the current evidence remains equivocal [89]. The anti-inflammatory effects of exercise may also be mediated by increased insulin sensitivity and oxidative capacity, HDL cholesterol, and improved endothelial and autonomic nervous system functioning. Thus, it is clear that exercise induced reduction in CVD risk is related to multiple mechanisms that are unlikely to operate in isolation. Recent advances in metabolomics platforms may help to further elucidate biological signatures associated with physical activity [90].

Table 1.1 Goals and recommendations for physical activity and cardiovascular disease risk

Goal	Evidence statement	Evidence[a] Type	Strength
Minimal benefit			
At least 150 minutes of moderate-intensity aerobic activity per week	Physical activity is inversely associated with cardiovascular disease risk	C	1
	Physical activity acts favourably on low grade inflammation and haemostasis	B	3
	Physical activity acts favourably on lipid profiles	B	1
	Physical activity reduces blood pressure	B	1
	Physical activity improves cardiorespiratory fitness	B	1
Greater benefit			
Performing some vigorous-intensity aerobic exercise on a weekly basis	Vigorous exercise confers greater protection against cardiovascular mortality, especially in men	C	2
Moderate- to vigorous-intensity activity	Moderate-vigorous activity confers 'optimal' benefits for blood pressure lowering, haemostasis and lipid levels	B	3

[a]Type of evidence: (A) major randomized, controlled trials (RCTs); (B) smaller RCTs and meta-analyses of other clinical trials; (C) observational and metabolic studies; (D) clinical experience. Strength of evidence: (1) very strong evidence; (2) moderately strong evidence; (3) strong trend

5 Summary and Conclusions

The Table 1.1 presents evidence statements based on a summary of the evidence given in this chapter. The evidence is such that exercise has been described as today's best buy in public health [91]. It's time to take physical inactivity seriously and we agree with those who would have policies to increase both physical activity and cardiorespiratory fitness [43, 48].

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Part II
Cardiac Exercise Physiology

Chapter 2
Acute and Chronic Response to Exercise in Athletes: The "Supernormal Heart"

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Abstract During last decades, most studies have examined the exercise-induced remodeling defined as "athlete's heart". During exercise, there is an increased cardiac output that causes morphological, functional, and electrical modification of the cardiac chambers. The cardiac remodeling depends also on the type of training, age, sex, ethnicity, genetic factors, and body size. The two main categories of exercise, endurance and strength, determine different effects on the cardiac remodeling. Even if most sport comprise both strength and endurance exercise, determining different scenarios of cardiac adaptation to the exercise. The aim of this paper is to assemble the current knowledge about physiologic and pathophysiologic response of both the left and the right heart in highly trained athletes.

Keywords Athletes heart • Doppler • Exercise-induced cardiomyopathy • Left ventricular hypertrophy • Right heart • Sport training • Strain

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Abbreviations

MET	metabolic exercise training
LV	left ventricle
RV	right ventricle
LA	left atrium
RA	right atrium
AoR	aortic root
ECG	electrocardiography
HCM	hypertrophic cardiomyopathy
DCM	dilated cardiomyopathy
ARVC	arrhytmogenic right ventricular cardiomyopathy
TDI	tissue Doppler imaging
RBBB	right bundle branch block
TAPSE	tricuspid annular plane systolic excursion
PASP	pulmonary arterial systolic pressure
CMR	cardiac magnetic resonance
PTAC	pulmonary transit of agitated contrast
BSA	body surface area
LAVI	left atrial volume indexed
TNF-α	tumor necrosis factor-α
STE	speckle tracking echocardiography
BAV	bicuspid aortic valve

The physical exercise leads many benefits, especially, in reducing the cardiovascular risk and in improving quality and expectancy of life. It has been demonstrated that the regular physical exercise reduces of about 30% the cardiovascular risk in healthy people [1]. Moreover, long term regular exercise training determines, especially in elite athletes, several cardiac structural changes, which represent the "athlete's heart", characterized by bradycardic rhythm at rest and enlarged cardiac chambers [2].

The current recommended regular physical exercise of 150 min per week of moderate physical exercise is far from developing the athlete's heart. Its development occurs in athletes that regularly perform 20 h of intense exercise (15 METs) per week to participate to competitive races. Cardiac adaptations of the "athlete's heart" is affected by many factors as the ethnicity, genetic, age, sex, and type, intensity and duration of exercise. Physical activity determine an increased stroke volume and enhanced diastolic filling also at high heart rates, reduction of vascular resistance and of heart rate due to the improvement of vagal tone and reduction of sympathetic tone. Isotonic or endurance sports (for example walking, running, swimming and skiing) are characterized by aerobic work and long distance exercise. In acute phase of endurance exercise, there is an increase of cardiac output, maximum oxygen consumption and peripheral vasodilatation in order to satisfy the

oxygen demand of tissues [2]. Strength training (or isometric exercise, such as weightlifting, wrestling or throwing heavy objects) is an anaerobic exercise in which the muscle fibers retain the initial length during the exercise but contract to develop tension against the afterload increase. Moreover, it implies the improvement of strength, anaerobic work and dimension of skeletal muscles. The improvement of oxygen provision and of the cardiac output are not necessary during isometric exercise, that determine a prevalent increase of the blood pressure, heart rate and peripheral vascular resistance [2].

Long-term cardiovascular adaptation to endurance exercise produces increased maximal oxygen uptake due to increased cardiac output and arteriovenous oxygen difference, while strength exercise results in little or no increase in oxygen uptake [3]. Thus, endurance exercise determines a predominant volume overload and strength exercise a predominant pressure overload.

In this chapter, we describe separately the acute and chronic cardiac effects of physical exercise on the left ventricle (LV), the right ventricle (RV), the left atrium (LA), the right atrium (RA) and the aortic root (AoR). In the 1899 Henschen, by using only the physical examination, discovered that cross-country skiers had larger heart and concluded that the enlargement involved both the right than the left ventricle [3]. Today various techniques are available, but most of data about the adaptation of cardiac structures to the exercise derive from the color-Doppler Echocardiography and from the new diagnostic technologies such as Doppler myocardial imaging, two-dimensional speckle tracking echocardiography (STE) and cardiac magnetic resonance which are able to anticipate some modifications before they become evident to the standard echocardiography. The aim of the application of all these techniques to the study oh athlete’s heart is not only to describe the adaptation of the heart to the exercise, but also to differentiate this benign adaptation from pathologic conditions such hypertrophic (HCM), dilated cardiomyopathy (DCM) and arrhytmogenic right ventricular cardiomyopathy (ARVC).

Electrocardiography (ECG) is the first tool to examine the athletes, both the athletes with symptoms suggestive of cardiac disease than for the asyntomatic athletes in pre-partecipation screening. Adaptive ECG characterizes on healthy athletes tipically include sinus bradycardia, sinus arrhythmia, hearly repolarization pattern [4] and increased QRS voltage [5]. Other signs as left bundle brunch block, repolarization abnormalities such as ST-segment depression or T wave inversion and pathological Q waves are abnormal signs and are closely associated with underlining and occult cardiomyopathy- in these cases further diagnostic evaluation are necessary in order to make an accurate diagnosis. Between the normal and the pathological signs there are some one that are collocated in the “grey zone”. One of these is the right bundle branch block (RBBB), complete or incomplete. RBBB could be an early manifestation of cardiomyopathies that involves the right heart, such as the ARVC. But often it can be detectable in healthy athletes with a normal heart. It has been demonstrated that the presence of RBBB and its duration correlates with the biventricular enlargement and is associated with a reduced rest systolic function. These two aspects are frequent in healthy endurance training athletes [6]. Complete

or incomplete RBBB can be considered an adaptation finding of the athlete's heart if other pathological signs are absent. Inparticular the presence of T-Wave inversion in V1, V2 and V3 and Epsilon wave, which make the suspicion for ARVC.

1 Right Heart and Pulmonary Circulation

During exercise, the increased cardiac output of the LV determines an augmented venous return to the right chambers, which progressively enlarge during the physical exercise more than the LV to adequately collect the venous return. The enlargement of the RV developes during the endurance exercise. Also the diastolic function change, in fact it is demonstrated an increased atrial component of the pattern of flow across the tricuspid valve [7]. This volume overload determine both RA and RV dilatation and increased wall thickness. It has been described greater RV inflow and outflow dimension in the athlete's heart compared to sedentary controls, with a normal systolic function expressed by tricuspid annular plane systolic excursion (TAPSE) [7]. Moreover, the higher the level of trained athletes and most obvious is the heart adaptation. Baggish et al. studied a population of 40 athletes: 20 Olympic rowers and 20 university level rowers. Olympic athletes shown greater RV end-diastolic chamber dimensions and, at the same time an improvement of both systolic and late diastolic relaxation examined by color tissue Doppler (TDI) and strain analysis [8]. Recently, D'Andrea et al. described the distribution of dimensions of RV (and also the RA) in a group of athletes and the impact of the type of long-training on these variables. They observed an increased of the cavitary dimensions with higher RV sphericity index in endurance athletes [9]. Enlargement of RV dimension is associated with an improved diastolic function, with a normal systolic function. Moreover, in the "athlete's heart" the LV stroke volume and pulmonary artery systolic pressure (PASP) are predictors of RV dimensions, demonstrating that there is a high interdependence of the two ventricles. The alternative method to evaluate the RV is the cardiac magnetic resonance (CMR) expecially for athletes who have a poor acoustic window. This diagnostic tool has a high spatial and temporal resolution. It is accurate for measuring the wall thickness and for the tissue characterization, that is very important to differentiate the physiological enlargement of the athlete's heart from pathological conditions as ARVC [2].

1.1 Acute Changes in Right Ventricle in the Post Exercise Phase

The effects of physical exercise on the RV are just evident in the acute phase post-exercise. Right ventricle is the first chamber to show the adaptation of the athlete's heart to exercise expecially in the phase post-exercise during endurance training.

Recent studies demonstrated a significant reduction of RV fractional area change of 12–32% and of the TAPSE of 4–22% during this phase [10]. Because these parameters could be influenced by the load conditions, it has been studied the RV free wall strain and the strain rate after exercise because they may be less influenced by load conditions. As a result, a reduction of 15% has been reported in post exercise RV strain, while changes in RV strain rate are more variable, probably because the strain rate is more dependent by the load condition [10–14]. The fact that RV strain rate has been observed to reduce in some studies but not in others may due to the variability of this measure. If it is real that RV strain rate is modified by load condition, also the LV strain rate should be modified by load condition. However, some studies have reported that the RV strain rate reduces while LV strain is preserved [10–13]. Previously, 20 years ago, Douglas et al. have just demonstrated that after an ultra-endurance triathlon, RV dilatation appears immediately whereas the LV dimensions did not change [15]. During the phase of diastolic overload of RV the interventricular septum is pushed towards the LV determining an increase of the LV eccentricity index [10, 12]. These observations are also demonstrated with the CMR performed post-exercise [16, 17]. The RV is the cardiac chamber that disproportionately suffers the fatigue during an intense endurance exercise. The explanation of this work overload on the RV can be found in the fact that endurance exercise is an aerob work that requires greater oxygen supply to the muscle tissue and, so cardiac output have to increase five to eightfold. This is obtained with an enhanced venous return, increase of myocardial contractility and dilatation of pulmonary arteries. During exercise, PASP increases proportionally with the cardiac output. La Gerche et al. performed echocardiography monitoring during exercise and found a greater increase in PASP than in invasively measured systolic blood pressure. They demonstrated that RV wall stress and work is much more high than the LV work during endurance exercise [18, 19]. This analysis is conducted with a non-invasive method to estimate the PASP. Other studies have evaluated the invasive measure of PASP with catheterization of the pulmonary arthery, demonstrating the same linear relationship between cardiac output and PASP and the marked increase of PASP during exercise in athletes. Moreover, La Gerche et al. demonstrate that the increase of PASP during exercise is associated with the enhancement of pulmonary vascular reserve, evaluated through the study of the pulmonary transit of agitated contrast (PTAC) to [20]. At rest, agitated echicardiographic contrast does not significantly pass through the pulmonary circulation at rest, but may do so during exercise. The researchers demonstrated that, during exercise, the PTAC was higher in the subjects with higher PASP and lower pulmonary vascular resistance and that this result did not depend by training status. During intense exercise of short duration, there is an increased RV function and volume work overload, but it is able to recovery completely at the end of training. It remains to be determined whether recovery of intense exercise-induced RV dysfunction is complete in all athletes from repeated bouts.

1.2 Chronic Changes in Right Ventricle in the Long Term Exercise

RV adaptations are more evident in endurance exercise that determines an eccentric remodelling. RV dimensions appear greater in endurance athletes of high categories than in strength athletes and sedentary controls when analyzed after a long term training. RV free walls appear thicker in endurance athletes (normal value of RV free wall thickness is inferior to 0.5 cm, measured with echocardiography from the sub-costal or parasternal long axis view). The inferior vena cava is larger (average value 26 mm, upper value 40 mm) but with a normal collapsibility in inspiration [2]. In a recent meta-analysis study, it has been demonstrated a positive and significant correlation between body surface area (BSA) and RV parameters. For this reason, it is necessary to index RV parameters for BSA in athletes [21].

Using monodimensional or bidimensional mode 2D imaging, the thickness should be measured at end-diastole at the level of tricuspid valve chords, excluding the thickness of the papillary muscle. Other necessary measures to evaluate the RV enlargement are the RV basal diameter (RVD1), RV medium-ventricular diameter (RVD2) and RV base-apex diameter (RVD3) in apical four chamber view. A recent experts consensus on echocardiography on athlete's heart have proposed some range values for RV in athletes [2] (Table 2.1). Several cohort studies have demonstrated augmented systolic function in athletes using the TAPSE as a measure of global RV function [22]. However, a large study recently showed that echocardiographic systolic parameters of RV systolic function were slightly reduced in athletes at rest compared to nonathletic controls. This reduction was more pronounced in those with more evident RV dilatation. D'Andrea et al. have evaluated the RV systolic function in 430 athletes by using both 2D and 3D echocardiography. They have demonstrated that all 2D RV diameters and 3D volumes were higher in endurance

Table 2.1 Upper range for cardiac ventricles dimension in athletes [48]

	RVD1(mm)	RVOT (mm)	LVEDD (mm)	LVWT (mm)
Caucasian adult				
Male	55	43	63	12
Female	49	40	56	11
Caucasian adolescent (14–18 years)				
Male	–	–	58	12
Female	–	–	54	11
Black adult				
Male	55	43	62	15
Female	49	40	56	12
Black adolescent (14–18 years)				
Male	–	–	62	15
Female	–	–	56	11

LVEDD left ventricular end diastolic diameter, *LVWT* left ventricular wall thickness, *RVD1* basal right ventricular diameter, *RVOT* right ventricular outflow tract

athletes versus controls, while all 2D and 3D systolic indexes were comparable in both group [2]. Therefore, a "mild" reduction in RV function can be considered a physiologic adaptation. It can be explained by the increased end diastolic volume with a normal stroke volume that cause a reduced ejection fraction [2]. Anyway, a severe reduction in global systolic function is not present in the athlete's heart and should be considered as a pathologic condition.

In the athlete's heart it is very frequent the presence of a tricuspid valve regurgitation on Color Doppler analysis in presence of normal valve leaflets and anulus. It is often mild and it is a consequence of the enlarged size of right chambers. The regurgitant jet is typically central and the PASP upper limit is 40 mmHg [23]. More often the higher values of PASP are found in endurance athletes rather than strength ones. Moreover, it has been found that the LV stroke volume is an independent predictor of PASP, that, in presence of normal pulmonary vascular resistance, can be considered as a "physiological phenomena" of physical exercise.

The regional systolic function has been also evaluated using tissue deformation imaging. In endurance athletes, both TDI and 2D–strain-derived parameters show significant difference respect to healthy sedentary people [24]. The systolic deformation of the inlet, the basal portion and, to a lesser extent, of the mid free wall of the RV, is significantly lower than the same region in non-athletes. La Gerche et al. demonstrated that this regional systolic wall anomalies at rest normalize itself at the peak of exercise, demonstrating a preserved contractile reserve. This observation suggests that the wall abnormality of regional systolic function at rest are not the result of a ventricular damage, but a physiological adaptation in response to RV dilatation [25].

About the RV diastolic function, there are non univocal data. Some authors have reported increased filling pressure of the RV [22, 26, 27], while others found no change compared with nonathletic controls [28, 29]. Tissue Doppler velocity measurements reveal an accentuated early-diastolic phase of the ventricular filling and prolonged isometric relaxation time, similar to those described for LV. Indeed, the time of regional release (RTm) and the velocity of early diastolic filling (Em) of the free wall of RV correlates with stroke volume of LV, demonstrating an interdependent relation between the two chambers. RV RTm is prolonged providing an optimal diastolic filling in order to reach a greater right systolic stroke volume. At the same time, the augmented stroke volume of the LV determines an increased venous return towards the right chambers with a higher flow that causes a prolongation of the RTm. About the decline of diastolic function of RV in athletes, it has been demonstrated that only the age and heart rate, and no other factors, such as amount of endurance exercise, influence the diastolic parameters. So if an altered diastolic function of RV is founded in a young athlete, it could indicate underling heart disease and require further investigation [30].

2 Right Atrium

The normal RA is an oval chamber that supports the filling of the RV and represents a "passive conduit" to the RV in early diastole. It completes the diastole with an active contraction, that contributes to 30% of RV cardiac output. During physical exercise, there is a volume overload of the right chambers that involves also the right atrium [9]. The strength exercise generate an acute pressure overload with only transient increased of volume overload of RA, that normalize at rest. Instead, during endurance exercise, the volume overload causes an acute increase of RA dimension that persists also at rest in the athlete who perform endurance exercise at competitive level for long term period. This different mechanism explains the difference of the dimensions of RA in endurance athletes, often higher than in strength athletes. Acute, transient RA (and RV) dilatation correlates with the release of cardiac markers of workload such as B-type natriuretic peptides [10] and cardiac troponin-I [17], immediately after severe endurance exercise such as marathon running. Several observational studies demonstrate a prevalence of RA dilatation that is independent on age [30]. In a cohort of more than 1.300 elite athletes, it was observed a prevalence of RA ECG abnormalities (P-wave amplitude more than 2.5 mm in the inferior leads) in 1.2% of endurance athletes and 0.5% in non-endurance athletes [31]. For echocardiographic evaluation of RA size, it is recommended to measure the RA area, because this parameter is easier to obtain and seems more reliable rather than the RA diameters or volume [32, 33]. The American Society of Echocardiography proposed a cut-off of 18 cm^2 for the RA dimension, but this value is derived from a study of small dimension and is not indexed for age, BSA or gender [34].

The largest observational studied was realized by Ekkehard et al. [32]. They prospectively analyzed the RA dimensions of a population of 880 Caucasian healthy subjects (composted of non athletes, strength and endurance athletes), with the aim of defining the mean value and the cut-off of RA dimension. They measured the RA area at the end of ventricular systole (when atrial chambers reach the maximum size), by using a 2 dimensional echocardiography in four-chamber view.

It was observed that RA mean area was similar in non athletes (12.5 ± 2.0 cm^2) and in strength athletes group (12.7 ± 1.6 cm^2), with a superior cut-off area for both group of 15 cm^2. In endurance athletes the RA area was higher (15.4 ± 2.1 cm^2) with a superior cut-off area of 18 cm^2. Data were also stratified for gender, age and BSA. It was found that BSA is the second determinant of RA dimension, after the type of sport. The gender also determine difference in the RA area, with higher value in men (Fig. 2.1), even if this difference disappears when the values are indexed for BSA (Table 2.2). It was hypothized that also the race would determine differences on the RA dimension. At this purpose, Zaidi et al. compared the RA dimension in a group of about 300 black athletes with a group of 375 white athletes and any difference was found [35]. This indicates no need to obtain cut-off value of RA stratified for race, but only for type of sports and BSA.

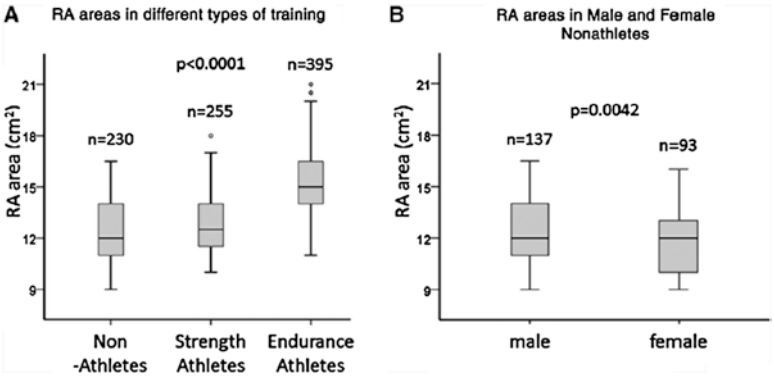

Fig. 2.1 Determinant factor of RA area. (**a**) type of training. (**b**) Gender [32]

Table 2.2 Mean absolute and for body surface area (BSA) of the right atrium (RA) [32]

		RA area (cm^2)			RA area Index (cm^2/m^2)		
	Number of subjects	Mean ± SD	Q-95	Q-95 (L-CI/U-CI)	Mean ± SD	Q-95	Q-95 (L-CI/U-CI)
Men							
Non-athletes	137	12.5 ± 2.0	15.7	15.1–16.5	6.7 ± 1.0	8.4	8.1–8.8
Strength	155	12.7 ± 1.6	15.3	14.8–15.9	6.9 ± 0.8	8.3	8.0–8.6
Endurance	255	15.4 ± 2.1	18.9	18.4 ± 19.5	8.3 ± 1.1	10.1	9.8–10.4
Women							
Non-athletes	93	11.9 ± 1.9	15.1	14.3–16.0	6.5 ± 1.0	8.2	7.7–8.6
Strength	100	12.8 ± 1.5	15.3	14.7–16.0	7.0–0.8	8.3	8.0–8.6
Endurance	140	15.3 ± 2.1	18.7	18.0–19.5	8.2 ± 1.1	10.0	9.6–10.4

3 Left Atrium

LA has an oval shape and is located in the back position of the other chambers in the mediastinum. The adaptation of athlete's heart also involves the LA, that presents larger dimension in the athletes, because LA pressure and volume increase during exercise [33]. In athletes, LA adaptation is not isolated but always associated with LV cavity enlargement. During ventricular diastole, the LV pressure is transmitted back to the LA. During diastole LV filling pressure increase and it is necessary that also LA pressure rises to obtain a complete LV filling. This mechanism determines LA enlargement and stretch on atrial cardiomyocites. The imaging technique used to study LA dilatation and function is the standard transthoracic-echocardiography with color Doppler and the novel techniques such as Doppler Tissue Imaging (DTI), STE and 3-dimensional echocardiography. The novel DTI and STE allow to evaluate the atrial myocardium remodeling before the eventual occurrence of dilation

[36, 37]. They have been also applied to the study of LA remodeling in athletes. The aim of this technique is to distinguish the LA adaptation secondary to left ventricular hypertrophy due to the exercise from pathologic cardiac remodeling due to arterial hypertension, diabete or cardiac valvular diseases.

In a cohort study of 1300 elite athletes, it was observed a prevalence of LA ECG abnormality (expressed by P-wave duration more than 120 msec in I or II leads with a negative deflession of the P-wave greater than or equal to 1 mm in depth and 40 msec in duration in V1 lead) in 1.2% of endurance athletes and in 0.5% of non-endurance athletes, respectively [31]. The presence of this abnormality requires a further investigation with echocardiography.

By using 2D echocardiography, the LA size should be measured at the end of ventricular systole, when it reaches its greater dimension. The easier method to evaluate the LA dimension is the measurement of the antero-posterior diameter (in the parasternal long axis view), the longitudinal and the trasverse diameter (in apical four chamber view) [38]. Linear dimension are simple to obtain but considered inaccurate, so measurement of volume rather than linear dimension or area is preferred [38, 39]. Measurements of LA volume (LAV) is obtained by ellipsoid model and Simpson method, measuring the LA area and the longitudinal diameter in apical four-chamber and apical two-chamber views [38].

LAV should be indexed for the BSA to obtain the Left Atrial Volume indexed (LAVi) [33]. The cut-off to define LA enlargement is established to 34 mL/m^2 as indicated in the ASE/EAE guidelines [2, 38]. Anyway this cut-off has been calculated on a large non-athlete population.

Pelliccia et al. conducted a large study on 1777 competitive athletes and found a small increase of LA antero-posterior diameter($\geq$40 mm) in 18% of athletes and a greater dilatation ($\geq$ 45 mm) in 2%, that was proportional to the LV cavity enlargement. As the 20% of athletes have a dilatation, it means that a mild enlargement is a physiologic adaptation to exercise [39]. For this reason, it was established the upper limit to 45 mm in female and 50 mm in male athletes to define LA enlargement evaluated with the linear method [2, 39] (Table 2.3). Regards to the LAVi dimen-

Table 2.3 Athlete's left atrial morphological and functional parameters

Authors	Number of athletes	Type of exercise	Parameter	Mean value	Upper value
Pelliccia et al. [39]	1777	Endurance or power	LA diameter (male) (mm)	37	50
			LA diameter (female) (mm)	32	45
D'Andrea et al. [40]	615	Endurance or power	LA volume index (male)(ml/m^2)	28	36
			LA volume index (female) (ml/m^2)	26.5	33
D'Andrea et al. [43]	80	Power	LA strain (%)	50	80

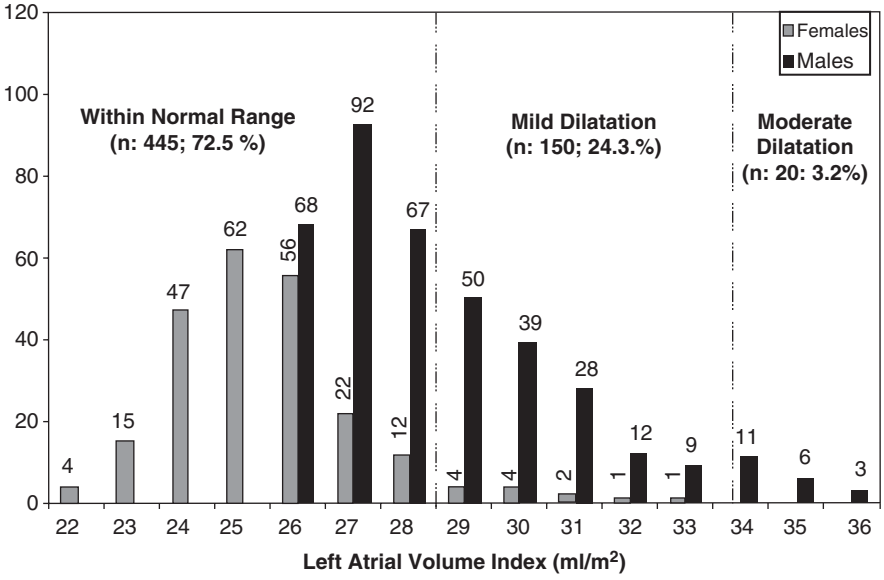

Fig. 2.2 Distribution of LA volume index in 615 athletes [40]

sion, in a recent observational study, D'Andrea et al. [40] examined 615 trained athletes and found LA mild enlargement (LAVi 29–33 ml/m^2) in 150 athletes (24.3%) and a moderate enlargement (LAVi ≥34 ml/m^2) only in 20 (3.2%) of athletes (all were men). The upper limit was 36 ml/m^2 (Fig. 2.2, Table 2.3).

The type and duration of training are the most important independent predictive factors of LAVi. In fact LAVi is significantly greater in endurance athletes. Because of a mild or moderate dilation is frequent in athletes, a cut-off of 36 ml/m^2 may help to identify the athletes with an abnormal LA dilation who require further investigation [2, 40]. This cut off could also help to avoid to categorize erroneously a LA dimension as abnormal for an athlete [2]. Thus, LA enlargement is a physiological consequence of the enlargement of all cardiac chambers. Another study confronted the LA dimension between athletes and a group of sedentary healthy control and found that the LA enlargement (LAVi ≥34 ml/m^2) has a prevalence of 67% in athletes [41]. Moreover, the major determinants of LAV in athletes are LV and-diastolic volume index, age and LV mass, while in non athletes the major determinants are the body mass index and the E/A ratio [30, 41]. In patients with a suboptimal acoustic window, CMR is a rationale alternative to study LA dimension. It provides great anatomic detail and also the presence of LA wall scar, for example after a radiofrequency ablation. Even if little data are available, also this technique has demonstrated LAV enlargement in athlete's heart. However, atrial volumes normalized for total heart volume do not differ between athletes and controls, indicating that LA enlargement is balanced on the total heart volume enlargement [42]. Of interest, the female athletes have smaller LA dimension, confirming the results of the previous studies performed with echocardiography [42].

LA function is an important part of cardiac cycle to determine an adequate LV filling. In a study conducted by D'Andrea and collegues [43], it has been demonstrated that the atrial function, expressed by LA strain is normal in elite athletes compared with sedentary healthy controls and hypertensive patients, all age-matched (Table 2.3). Atrial longitudinal strain was performed from the apical four and two chamber views for the basal segment of LA septum, lateral wall and roof. LA diameter and maximum volume were increased but similar in both groups. The peak systolic myocardial atrial strain was significantly reduced in patients with pathologic LV hypertrophy compared with controls and athletes in all studied segments.

In a multivariate analysis, LV end-diastolic volume and LV mass were the major predictive factors of LA lateral wall peak systolic strain. Instead, it was found a negative correlation of LA lateral wall peak systolic strain with LV mass and circumferential end-systolic stress in subject with hypertension [43]. Therefore, LA myocardial deformation is normal in elite athlete's compared with aged-matched sedentary controls and hypertensive patients. Thus, the atrial enlargement oh athlete's heart does not mean loss of atrial function, but, conversely, it indicates an increase of functional capacity during exercise.

The LA enlargement is considered a risk factor for atrial fibrillation (AF) in general population. Several studies have analyzed the relationship between long-term endurance sport and AF. Firstly, Karjalainen and collegues [44] published in 1998 a prospective longitudinal study in which after 10 years of follow up, AF incidence was 5.3% among athletes compared with 0.9% among the control subjects. After, Molina and collegues [45] analyzed the incidence of AF in a group of 183 amateur marathon runners and in a group of 290 sedentary controls. The annual incidence was 0.43% in the former and 0.11% in the latter group. Moreover the risk of AF correlates with hours and intensity of exercise [46]. On the other hand, Mozaffarian and collegues [47] demonstrated that a daily and costant exercise, such as walking, is associated with significant lower AF incidence in older adult, while a more intense physical exercise is associated with a moderate increase of risk of AF. Some studies have defined an exercise risk threshold for developing AF. They reported that a lifetime exercise practice of more than 5000 h and formore than 5 h per week at the age of 30 years or over increases the risk of AF [48]. The mechanisms of lone AF in the athlete's heart are various: LA remodeling, increased vagal tone and atrial ectopic beats. Fibrosis is a common feature of atrial remodeling in some pathologic conditions, such as hypertension. There are little data on the atrial wall fibrosis in humans. In rat models of marathon running, it has been demonstrated a profibrotic process in rats subjected to endurance exercise. Endurance exercise is associated with acute post-exercise release of inflammation and oxidative factors, which favour the development of wall fibrosis [49–51]. The most studied factor is the tumor necrosis factor-α (TNF-α) [51]. Data of the preclinical studies demonstrate that the atrial fibrosis is not sufficient to determine AF, but other factors are necessary. For example, the increased vagal tone favours the excitability of myocardium creating the substrate for a re-entry circuit [52] while the atrial ectopic beats could be the trigger to start the arrhythmia. Therefore, the sport-related AF may occur in a middle-aged male athlete with a history of long-term regular endurance sport practice, expecially

involved in high endurance training [30, 53]. Tipically, this arrhythmia is paroxysmal self limiting AF and occurs during the night or after meals, demonstrating that the vagal overtone is an important trigger.

4 Left Ventricle

In the last 35 years, the development of echocardiographic technique has allowed to study the adaptation of the LV to physical exercise in order to discriminate the physiological adaptation from the patholoyhcal changes. The two principal categories of exercise (strength and endurance) determine different adaptations of the LV, as hypothized by Morganroth [54]. According to this hypothesis, endurance training would lead a volume overload and, so to an increased diastolic wall stress. The adaptation of the LV to this work is eccentric ventricular hypertrophy (increase of both ventricular mass and ventricular cavity dimension). In contrast, strength training determines pressure overload and increased systolic wall stress. In this case the answer of the LV is a concentric hypertrophy (increase of ventricular mass and of wall thickness with normal cavity dimension). Both of them result in increased LV mass. LV hypertrophy is definided by a LV mass index >115 g/m^2 in male and >95 g/m^2 in female. The relative wall thickness (2 × posterior wall thickness/LV internal end-diastolic diameter) defines the type of hypertrophy: eccentric when the relative wall thickness is ≤0.42, concentric when the relative wall thickness is >0.42 [55].

The "Morganroth Hypothesis" has some limitations because some types of sports, such as cycling and rowing, imply both endurance and strength exercise, and the hypertrophy results in an intermediate phenotype (Fig. 2.3). Furthermore, especially with strength exercise, the phenotype could be not completely explained because of the confounding factor of the drug abuse (for example steroids).

Generally, athletes show a 10–20% increase of the wall thickness and 10–15% enlargement of the cavity [48] compared with individual of similar age and size. The LV enlargement is always proportionate to the enlargement of the other cardiac chambers. Adaptations of right chambers to the physical exercise are visible right after a prolonged physical training, instead the LV adaptation to the exercise become visible after a period of training of several months. In fact in adolescent athletes, the magnitude of this modifications is lower because of the shorter period of training [56–58]. The LV adaptation regresses after a period of de-training of about 3 months. After this "physical de-conditioning", it has been demonstrated a reduction of 15–33% of the septal wall thickness, whereas the reduction of both septal thickness (of about 15%) and LV cavity dimension (of about 7%) may be observed after a period of 1–13 years of de-training. This demonstrated that the LV cavity reduce more slowly and slightly than the LV wall thickness. The regression of the "athlete's heart" allows to make differential diagnosis with the pathologic hypertrophy (DCM and HCM). In these cardiomyopathies, the hypertrophy do not decrease after a period of de-training. Other aspects of the cardiomyopathies help to make

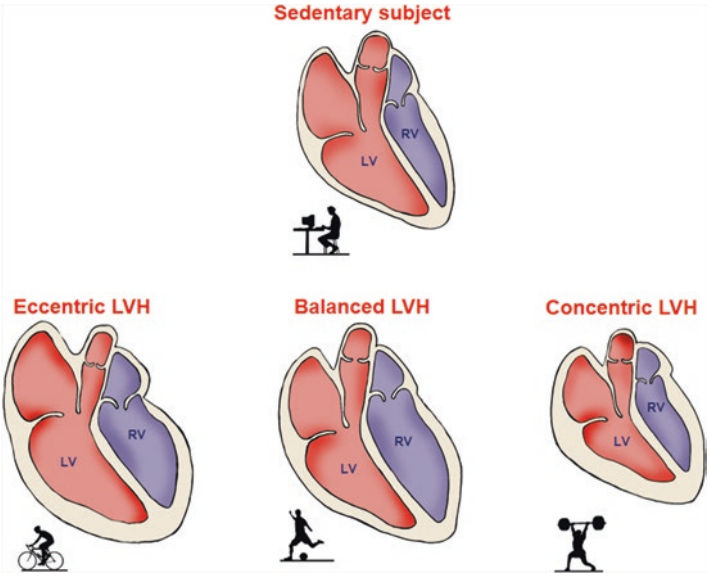

Fig. 2.3 Different models of left ventricular hypertrophy (LVH) secondary to long-term sport training. Endurance sports are associated with eccentric LVH, while power training usually determines concentric LVH. However, most of team sports are related with balanced form of LVH, with consequent right ventricular (RV) enlargement

the correct diagnosis. If HCM is suspected, some criteria should be considered as the presence of left outflow tract obstruction, systolic anterior movement of mitral valve, diastolic dysfunction and the family history. For suspected DCM, impaired or borderline systolic function and a peak oxygen consumption inferior to 50 ml/min/kg (<120% of the predicted) should be considered to make the differential diagnosis [3].

The pattern and the magnitude of LV mass may depend on the nature of the sports [3] (Fig. 2.3). Sports as cycling, rowing and swimming determine the major variation of both LV cavity and thickness, whereas athletes participating in ultra-endurance sport (as thriathlons) paradoxically show more modest alterations in cardiac dimensions, even if there are very limiting data [3].

Data assembled in a large populations of trained athletes assessed with multivariate analysis demonstrated that about 75% of variability in LV cavity size depends on non-genetic factors such as body size, type of sports, gender and age, with the BSA as the principal determinant. The remaining 25% of variability is not completely explained, but maybe genetic factors and the race play an important role. Because the BSA is the most powerful predictor of LV cavity dimension, the measures have to be always indexed for BSA. Larger athletes generally show greater absolute LV cavity and thickness dimension, that normalizes when corrected for BSA [3] (Fig. 2.4).

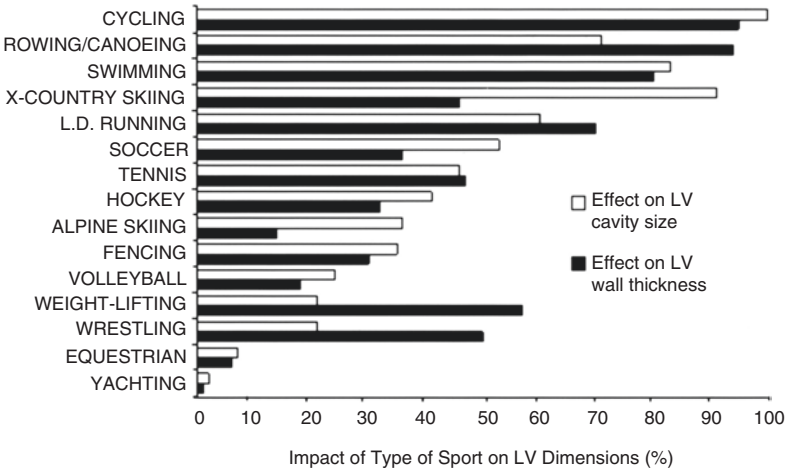

Fig. 2.4 Effect of different of 27 different sports on the LV cavity dimension and wall thickness in elite athletes. *LD* long distance [3]

In a group of 1309 athletes of different disciplines, 55% had an increased LV end-diastolic diameter and 14% of endurance athletes show values more than 60 mm, that is compatible with DCM. However, the presence of a preserved LV systolic function and a normal VO2 max during cardiopulmonary test may help to exclude DCM [2].

In 947 elite athletes, maximal end diastolic septal wall thickness was ≤12 mm. Only 1.7% had septal thickness ≥13 mm (range 13–16 mm). Septal thickness was lower in women (mean value 9 mm; upper limits 12 mm). The black athletes show often major LV wall thickness, with always a normal LV cavity, demonstrating that the race is a predisposing factor to an increased wall thickness rather than to an increased LV cavity [2]. LV hypertrophy with LV wall >12 mm is described in 13% of black male athletes and in 3% of black female athletes. Anyway, a wall thickness > 16 mm is very uncommon, irrespective of the race [3]. A recent interesting review has proposed the upper limits of the LV dimension, stratified for age, sex and race [48] (Table 2.1).

In athletes, the LV mass increase is always associated with a normal systolic function. The ejection fraction is normal with an increased stroke volume at rest, as a result of the increased pre-load (major end-diastolic diameters). By using TDI method, also the systolic peak velocity of the lateral annulus of mitral valve (s') is demonstrated to be normal or supra-normal. It has been proposed a s' cut- off <9 cm/s in order to distinguish the pathological hypertrophy (HCM or Hypertensive Cardiomiopathy) from the adaptation of the athlete's heart [2].

The athletes LV diastolic function is normal or even super-normal at rest, in particular in endurance athletes. By using Pulsed-Doppler echocardiography, the transmitral flow velocity expressed by the ratio E/A is >2 in athletes, because the large LV volume of refill increases the contribution of early diastolic phase to during the LV diastole. Instead, in pathological forms of LV hypertrophy the diastolic function

is impaired (E/A < 1 with a prolonged deceleration time). By using Pulsed TDI, early diastolic velocity (e') and the ratio e'/a' of basal septal and basal lateral wall are increased in athletes. Moreover, it has been demonstrated that LV inferior wall e' correlates with LV end-diastolic diameter demonstrating that in endurance athlete the LV cavity enlargement induces a proportional improvement of LV relaxation [2]. In endurance athletes, the novel technique of STE have been also used to analyze the LV function in athlete. In a group of professional soccer athletes it was found a reduction of global longitudinal strain at rest that is compensated by an increased radial and circumferential strain [59]. However, it has been described lower apical radial strain and lower twisting at rest in cyclists and not in sedentary controls [2]. These studies demonstrate that the athlete's heart adaptation at rest is different from that of sedentary controls and also that is loading dependent. Moreover, different type of sports determine different pattern of strain modification (Fig. 2.5).

There are many data of LV chronic response to exercise, but about the adaptation of left ventricle in the acute phase of post exercise, there are few and conflicting data. Immediately after an intense physical exercise, it is reported an increase of both Troponin and Brain Natriuretic Peptide that indicates a "cardiac fatigue" [16, 17].

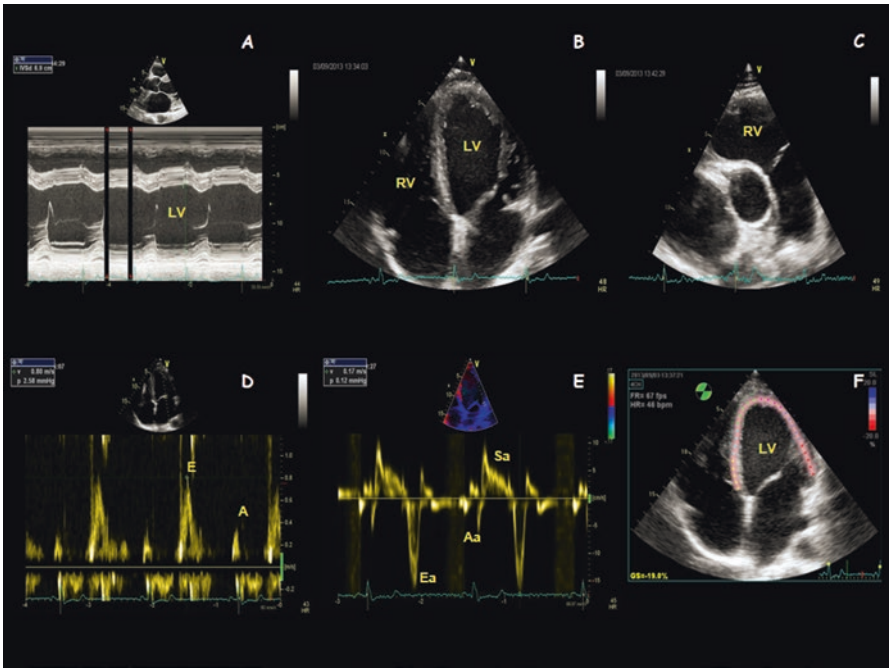

Fig. 2.5 Echocardiographic analysis of top-level endurance athlete, showing increased left ventricular cavity by M-mode (*A*) and B-mode, enlarged right ventricular (*RV*) outflow tract (*C*), super-normal diastolic function both at global (*D*) and regional (*E*) level, and normal myocardial deformation by global strain analysis (*F*)

A meta-analysis of 23 studies demonstrated a slight reduction of ejection fraction (−2%), of uncertain clinical significance that is, in part, explainable with a different load condition [21]. Some echocardiographic studies found wall regional abnormalities and reduced pulsed DTI e' velocity of both septal and lateral mitral annulus in runners after a marathon [2]. After long exercise, a decrease of longitudinal, circumferential and radial strain and a reduction and delay of peak twisting were also documented in thriathletes. Of interest, while the LV systolic function return to the normality in 2 days, the diastolic dysfunction persists until 1 months after a marathon. In some studies, the late gadolinium enhancement distribution on CMR is used to verify the presence of myocardial fibrosis in athletes as a sign of permanent injury. It was observed LGE in 12% of marathon runners and its prevalence was correlated with the number of marathons previously performed, suggesting that an intense training determine the development of small myocardial scars [60].

5 Aortic Root

Adaptations of athlete's heart involve also the aortic root (AoR). Endurance and strength exercise have different effects on the aortic root. During endurance exercise the increase of stroke volume repeated and protracted over time determines a major distension of the aortic wall and, so, a great systolic pressure during the exercise. The strength exercise is characterized by short exercise of high intensity that determine rapid and brief increase of cardiac output. At the same time, the enhanced sympathetic nervous system activity and external compression of blood vessels makes a rapid increase of heart rate and of the systemic peripheral resistance. Thus, during heavy-resistance static exercise, the arterial rapidly increase with values which arrive to 480/350 mmHg [61]. Starting from the pathologic model of aortic dilatation in arterial hypertension, it has been hypotized that the hemodynamic load during prolonged exercise and, particularly, the pressure overload during strength exercise may lead to AoR dilatation. A recent study [62] has explored the aortic root dimension on 615 elite athletes (370 endurance-trained athletes, 245 strength-trained athletes, with a mean age of 28.4 ± 10.2 years) using transthoracic-echocardiography (Table 2.4). The aortic root diameters were significantly greater in all segments in the strength-trained athletes with greater diameters in men than in women, even if this difference abolished when the data were indexed for BSA. Only

Table 2.4 Bi-dimensional echocardiographic root diameters in athletes (p value < 0.05) [62]

Variable (cm)	Overall (n = 615)	Endurance (n = 370)	Strenght (n = 245)
Aortic annulus	2.3 (1.8–2.8)	2.1 (1.8–2.4)	2.5 (2.2–2.8)
Sinuses of Valsalva	3.3 (2.8–4.2)	3.1 (2.8–3.6)	3.6 (3.2–4.2)
Supra-aortic ridge	3.1 (2.6–3.7)	2.9 (2.6–3.2)	3.3 (2.9–3.7)
Proximal ascending aorta	3.3 (2.8–3.9)	3.1 (2.8–3.4)	3.5 (3.1–3.9)

Data are presented as mean (range)

in 6 athletes (1%) an aneurism of aortic root was observed. A multiple linear regression analysis was made and it was demonstrated that BSA, type and duration of competitive training were the only independent determinants of AoR dimension.

A large meta-analysis of 23 observational study [63, 64] found that athletes have larger aortic root diameters compared to sedentary subjects, especially at the sinuses of Valsalva, that is statistically significant but not clinically significant. In this research, the 95% confidence limit was only 33 mm for men and 27.3 for women, respectively. Moreover, the physical exercise do not favour the enlargement of the aortic root also in patient with a risk factor such as the bicuspid aortic valve (BAV) but with normal aortic dimension [64]. It has been demonstrated that in 88 athletes with BAV the increase was 0.98 mm/year, the same rate observed in non-athletes with BAV (0.2–1.9 mm/year) [65]. These results demonstrate that the physical exercise determine a small enlargement of the aortic root, as a consequence of physical adaptation to the exercise, but it is never pathological and a marked enlargement (>40 mm) suggests a pathologic process, that could be exacerbated by physical exercise. Thus, it should be emphasized that subjects with aortic dilatation or predisposition to rupture (for example Marphan's syndrome) should be discouraged from practicing intense physical exercise (both endurance than strength).

6 Conclusions

Exercise practice, both for recreational and competitive purpose, is spreading worldwide. In fact, there are growing numbers of sports event (i.e., community-based road running races) and in the last years there is a greater awareness of documented health benefits.

Then, an increase in the number of subjects with features of exercise-induced cardiac remodeling could be expected. It is necessary for the cardiologist and sports medicine practitioners to possess at least a basic knowledge of this subject. With respect to the contemplated potential existence of an "exercise-induced cardiomyopathy" or of any potential excess of arrhythmias in athletes, it is critical to maintain some balance. There is a multitude of positive effects from exercise and these are very unlikely to be outweighed by any small risk of cardiac enlargement or arrhythmias. However, there is lack of knowledge on the potential negative effects on healthy of the "extreme" exercise. Data collection and new information should be taken in order to identify the threshold beyond which the exercise becomes no longer beneficial to health.

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Chapter 3
The Effects of Exercise on Cardiovascular Biomarkers: New Insights, Recent Data, and Applications

Lin Che and Dong Li

Abstract The benefit of regular exercise or physical activity with appropriate intensity on improving cardiopulmonary function and endurance has long been accepted with less controversy. The challenge remains, however, quantitatively evaluate the effect of exercise on cardiovascular health due in part to the amount and intensity of exercise varies widely plus lack of effective, robust and efficient biomarker evaluation systems. Better evaluating the overall function of biomarker and validate biomarkers utility in cardiovascular health should improve the evidence regarding the benefit or the effect of exercise or physical activity on cardiovascular health, in turn increasing the efficiency of the biomarker on individuals with mild to moderate cardiovascular risk. In this review, beyond traditional cytokines, chemokines and inflammatory factors, we systemic reviewed the latest novel biomarkers in metabolomics, genomics, proteomics, and molecular imaging mainly focus on heart health, as well as cardiovascular diseases such as atherosclerosis and ischemic heart disease. Furthermore, we highlight the state-of-the-art biomarker developing techniques and its application in the field of heart health. Finally, we discuss the clinical relevance of physical activity and exercise on key biomarkers in molecular basis and practical considerations.

Keywords Exercise • Cardiovascular disease • Heart health • Biomarkers

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1 Introduction

1.1 Biomarker—What Is Answered?

In the biomedical field, the biomarker is generally considered as a kind of bioindicator, which usually obtained from patient's bio-samples, and can be qualitatively or quantitatively measured by the clinical assay/testing equipment [1–4]. There are diverse categories of biomarkers per the source of the sample, application, assay methods, and even the stability of biomarkers. A biomarker can be gathered from the healthy person [5] and the sick patients' biological sample, for example, urine, blood, tissue biopsy specimens and so on [6–8]. Besides, a biomarker can be obtained from a clinical record, a combination of laboratory and clinical tests, for example, blood pressure, glucose and lipid components in serum, or biomarker can be gathered from imaging tests (ECG, echocardiogram, cardiac CT scan). To date, from the practical point of view, biomarker increasingly plays an important role in translating of highly promising basic research into clinical applications from a routine diagnostic test, therapy decision-making, and prognostic evaluation [9–11] (Fig. 3.1).

1.2 What Is an Ideal Biomarker?

Ideally, the biomarker should tightly associate with the different stages of the disease [12] or/and health status [13, 14], the intensity, and durability of physical exercise [15] such as physiological status, the stage of the diseases [16], the pathogenic

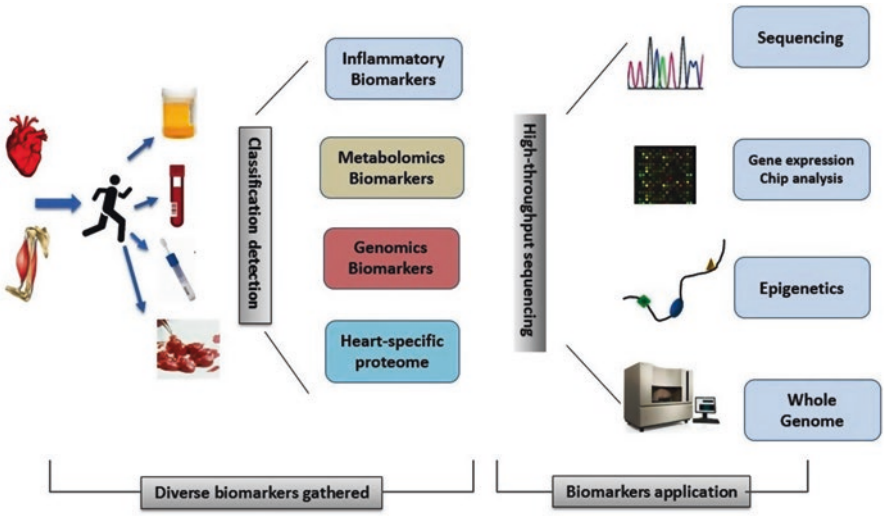

Fig. 3.1 Flowchart of biomarkers application in cardiovascular health evaluation

Table 3.1 Biomarkers: a basic glossary

Categories of biomarkers	subgroup	Discription
Inflammatory factors biomarkers	IL-6, IL-8, IL-10, TNF-alpha, hs-CRP, IL-6sR, IL-1sRII, sTNFRI, sTNFRII, IL-15, Adiponectin	Associated with exercise-induced cardiac dysfunction [20];
		Chronic heart failure [21]
Functional metabolomics biomarkers	1-naphthol, 2-naphthol, GlcNAc-6-P, L-carnitine methylitaconate, N-acetyl-d-glucosamine 6-phosphate and l-carnitine	Maximum oxygen uptake [22]
		Coronary artery calcium (CAC) progression [23]
The heart-specific proteome	Natriuretic peptide B, troponin T type 2 (cardiac), myosin binding protein C, ankyrin repeat domain 1, SH3 domain binding kinase family, myosin, light chain 4, alkali; atrial, embryonic	Interstitial fibrosis [24]

processes [17], the environmental exposure [18], the therapeutic intervention [19] and so on (Table 3.1). Additionally, an effective "ideal" biomarker has the following characteristics, according to the FDA: (1) Non-invasive/accessible. Ideally, the overall expectation of biomarkers, from the way in which the sample is obtained, the sampling should be non-invasive/accessible, or at least non-lethal. As a researcher or clinical physician, it is also important to consider whether samples are readily available, and the results obtained via a rapid access measurement or proposal. (2) high, sensitive and specific. Given one of the main purposes of the biomarker, applications are to make a definite diagnosis and to guide treatment methods and management strategies in clinical practice, beyond the rapid and inexpensive, high sensitive and specific is one of the important indicators. Applicable to a broad range of testing and lacking sensitivity and specificity is the primary reason restricting its application; (3) Cost effective. As an ideal biomarker, the overall costs should be reasonable.

2 Traditional Inflammatory Biomarker and Regular Physical Activity—at a Glance

The inflammatory biomarkers provide part of the comprehensive assessment of physical activity.

However, taking into account the diverse effect on physical activity and factor-mediated inflammation, in this part, we would like to concentrate on the applications and limitations of inflammatory biomarkers for the evaluation of some multi-center, community-based physical activity studies. Numerous studies have

reported the beneficial effects of physical exercise in the rehabilitation of the disease, in particular, on the heart [20, 21]. Beavers et al. tried to evaluate the effect of long-term (6–12 months) physical excise intervention on inflammatory biomarkers in the elder population [22]. In this study, a bundle of biomarkers includes a family of interleukin-related cytokines (IL-1ra, IL-2sRα, IL-6sR, IL-8, IL-1sRII, IL-15), adiponectin, and TNF- α were measured. They concluded that the IL-8 was the only inflammatory biomarker in their study population was affected by the physical activity. Similar results were observed in a short-term physical activity population. Lund et al. tried to investigate the stability of inflammatory biomarkers in short-term physical activity in middle-aged male [23]. Their data suggested that change the style of exercise (withdrawal of highly active and the imposition of daily exercise) will not elicit substantial changes in C-reactive protein (CRP), IL-6, and TNF-α. In other words, these inflammatory markers (CRP, IL-6, and TNF-α) are relatively stable and rarely affected by exercise behavior.

Notably, the latest data suggest that the inflammatory biomarkers may be affected by exercise intensity. Another study has different answers. Different from the previous studies, in this study, the authors selected the change of maximal oxygen consumption (VO_2max) as the primary outcome and tried to determine if the response of certain inflammatory biomarkers change (decrease or increase in the level of biomarkers) to the diverse of the exercise intensity [24–26]. The inflammatory biomarkers involved in this study include IL-1β, IL-6, IL-10, TNF- α, hs-CRP, soluble intercellular adhesion molecule-1 (sICAM-1), and ratios of TNF- α/IL-10 in circulating peripheral blood. The data clearly demonstrated that a single bout of exercise with high-intensity induces a transient increase in IL-6 and the ratio of IL-6/IL-10, and low-intensity can decrease the level of sICAM-1 [27].

3 Fibroblast Growth Factor 23 (FGF23): New Insight into Links Between Bone and Heart

Several initial studies indicated that fibroblast growth factor 23 (FGF23), a major regulator of phosphate homeostasis and vitamin D homeostasis, may play a unique role in linking benefits of physical exercise on the heart [28, 29]. FGF23 is a 32-kDa protein with 251 amino acids that is synthesized and secreted by bone cell (mainly osteoblasts) which was initially regarded as responsible for phosphate metabolism. Unexpectedly, a recent study indicated that FGF23 has significantly expanded into its present role as a key player in cardiovascular disease, though the study's sample size was very small [30, 31]. It will, nevertheless, be valuable to confirm these findings in additional settings, especially in athletes, fitness lovers or even community-based, multi-race/ethnic regular physical exercise population. The data about the FGF23 levels will itself be informative and be important in designing the future exercise evaluation, and the findings may act as a potential direction of benefit to the exercise on the heart function.

Though initial studies show that FGF23 is secreted from bone cells, and immediately circulated into peripheral blood to reach the whole-body [32–34]. FGF23 is regulated by bone proteins PHEX and DMP1 [35]. A recent study based on an animal model showed only chronic exercise or calorie restriction upregulates FGF23 mRNA and protein expression in skeletal muscle [36, 37]. FGF23 mRNA and protein expression may vary in different exercise patterns, or models, including acute exercise, exhaustive exercise, and chronic exercise. Li et al. used C57BL/6 J mice to evaluate the exercise performance, H_2O_2 production, reactive oxygen species (ROS), and functional mitochondrial biomarkers in muscle, the gene expression of sirtuin 1, and mitochondrial transcription factor A, PGC-1α, PPAR-δ, and citrate synthase activity was assayed. There have been some unexpected findings such as only chronic exercise upregulated the level of FGF23 mRNA and protein expression. In addition, the exogenous FGF23 can significantly extend the time to exhaustion, which associated with VO_2 and VO_2 max. As a matter of fact, this issue is extremely important to the evaluation of exercise promotes exercise performance by proteomics technologies, in particular for chronic exercise evaluation.

4 Troponins Proteins—A Novel Biomarker of Myocardial Injury in Physical Exercise

The benefit of physical exercise on heart function has been accepted with minimal debate. However, the intensity and amount of exercise vary widely, and another possibility exists, that is the physical exertion or exercise above a certain threshold may paradoxically worsen heart health. Thus far, some small studies from clinical have been performed that documented adverse cardiac and vascular events with high levels of endurance exercise. Investigating the potential role of prolonged exercise and endurance training elevations in adverse cardiovascular early reports focused on the serum concentrations of troponins proteins, a family of acidic regulatory molecules found in cardiac muscle. This phenomenon was partly due to cTns are highly specific biomarkers of response to myocardial cell damage [38–41]. So far, two high-sensitive regulatory proteins, which can be measured from serum, cardiac troponin T (cTnT) and troponin I (cTnI) have been found related to the mechanisms of actin-mediated regulation [42]. There is little good evidence from clinical data that cardiac troponins may as a potential biomarker in response to exercise [43]. The objective of this study was to determine if prolonged exercise resulted in the appearance of cardiac troponin T (cTnT) in serum and whether this was associated with elevated levels of myocardial oxidative stress. The initial evidence was derived from animal experiments [44]. Li et al. using a myocardial oxidative stress model built in Sprague-Dawley rats, their data clearly indicated that serum cardiac troponin T is significantly associated with the myocardial oxidative stress after prolonged exercise [45]. A pilot study from clinical trials leads by Lee et al., using a novel high-sensitivity cardiac troponin I (hs-cTnI) as a biomarker to investigate whether the

cTnI level in the peripheral blood is associated with exercise-induced myocardial ischemia [46]. With the help of the latest imaging technology (myocardial perfusion single-photon emission computed tomography), based on a total 819 patients with suspected myocardial ischemia induced by exercise, the author concluded that the exercise-induced myocardial ischemia significantly increased the hs-cTnI levels in these patients diagnosed myocardial ischemia as compared with those patients without myocardial ischemia conditions.

5 The Emerging Technologies— Influence Cannot be Ignored

It is well accepted that the physical exercise is an effective, as well as economic strategy to promote circulatory system health. Using biomarker to the accurate quantitative assessment of the intensity and durability of physical activity, meanwhile, to evaluate the effects associated with the benefits to the heart is highly desirable. With the growth of technological advances in the field of biomedicine, proteomics, as a powerful, cutting-edge subject that has enormous potential for evaluating the conditions and status of human health as well as physical activity. Below we summary some novel protein biomarkers' application.

5.1 NT-proBNP—A Bidirectional Impact Protein

Brain natriuretic peptide (BNP) is secreted by ventricular cardiomyocytes as a proBNP hormone. Recent findings from transgenic animal models indicated that overexpressing BNP have a fourfold increase [47] is associated with sharply adverse risk of cardiovascular risk factors [48, 49], cardiovascular disease (CVD) and heart failure [50, 51]. Using healthy controls, Aengevaeren et al. found the endurance exercise (30–50 km/week)-induced changes in BNP concentrations in cardiovascular patients versus BNP [52].

However, there are still unanswered questions concerning BNP's concentration in cardiopulmonary exercise. An extremely important study, led by Smart et al., meta-reviewed nine published studies (Cochrane Central Register of Controlled Trials, Embase.com (1974-current), CINAHL (1981-current), conducted of Medline (Ovid) (1950-July 2008), and Web of Science (2000-current)), they concluded that the exercise training had a mean decrease BNP 79 pg/ml (95% CI: −141, −17 pg/ml), as well as decrease NT-pro-BNP 621 pg/ml (95% CI: −844, −398 pg/ml) in patients with left ventricular dysfunction [53]. Although the exact mechanism remains unclear. These effects remind us that these biomarkers deserve further study.

5.2 *Serum Uric Acid (UA) Simple But a Long Neglected Biomarker*

Compared with other "novel" serum markers, the influence of exercise on serum uric acid has not attracted enough attention for a long time. Montoye et al. study based on a total of 4535 both genders, age range10–64 year's people. They found the serum uric acid is one of the good indicators which associated with one's physical fitness and is much correlated to body fatness than the response of heart rate on exercise [54, 55]. A similar result was observed in another race/Etcitty population. The authors selected categorized walking steps and the time per day as primary outcomes [56, 57]. These results are consistent with that serum uric acid may increase during conditions of high energy utilization. Additionally, our unpublished data based on an average 2.5 years follow-up data from a multi-center, large sample size cohort, also indicated that serum uric acid was associated with 1.40 (1.16–1.45) fold higher risk for adverse cardiac events even after additional adjustment for LDL, HDL, TG, Creatinine, BMI, and hypertension.

5.3 *How About Bioimaging?*

Traditionally, the physical activity always associates with bone structure and bone density change. Indeed. The bone mineral density (BMD) is evaluated by dual-energy X-ray absorptiometry (DXA) of lumbar spine and hip, with the use of other tests to assess atherosclerosis, when appropriate. This screening model adds considerable cost and patient burden but also radiation exposure. Additional, the sensitivity of two-dimensional (2-D) DXA techniques to accurately determine early bone loss is limited due to the natural overlapping formation of cortical and trabecular bone. Notably, trabecular bone (the metabolically active portion) is lost first and is the first to respond to medical therapies, making it a more realistic reflection of bone mineral metabolism and bone density status compared to cortical bone [58–60]. To date, the next-generation computed tomography (CT) scanner, MRI [61], particular cardiac CT scan provides better cardiac imaging [62–64], as well as vertebral bone mineral density information with minimal radiation, 92 of 2352 Olympic athletes, showed abnormal CV structure and arrhythmias [65]. Given bone density status and atherosclerosis, independent but highly interactive, we are cautiously optimistic that bioimaging marker (both quantitative and qualitative) gathered from next-generation CT scan has a potential to become a robust tool for exercise benefits evaluation. In fact, compared to DXA, cardiac CT, especially the latest generation CT, allows for a high-resolution three-dimensional (3-D) imaging by isolation of trabecular from cortical bone of the vertebral shell and posterior elements to assess true volumetric density, as well as cardiovascular function [66–68].

6 Concluding Comments

The biomarker has increasingly become a powerful research tool for assessing and monitoring physical excise, training status, and performance. Although considerable challenges exist, advances such as high-throughput screening platforms to screen gene expression, protein coding or even epigenetic modulation technologies become increasingly mature in the field of sports medicine. More importantly, compared with the traditional time-consuming and labor-intensive detection methods, modern biomarker system offers accurate, fast, reproducible, and highly sensitive high-throughput screening at a cost-effective price point. These innovations are necessary for big data-driven cutting-edge bioinformatics products which are emerging fields in sports medicine and biomarker research. We expect that the information we provided in this review will not only illustrate by summarizing existing knowledge and filling the gaps in knowledge but also *spark inspiration* for future heart-specific biomarker studies.

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Chapter 4
Acute and Chronic Exercise in Animal Models

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Abstract Numerous animal cardiac exercise models using animal subjects have been established to uncover the cardiovascular physiological mechanism of exercise or to determine the effects of exercise on cardiovascular health and disease. In most cases, animal-based cardiovascular exercise modalities include treadmill running, swimming, and voluntary wheel running with a series of intensities, times, and durations. Those used animals include small rodents (e.g., mice and rats) and large animals (e.g., rabbits, dogs, goats, sheep, pigs, and horses). Depending on the research goal, each experimental protocol should also describe whether its respective exercise treatment can produce the anticipated acute or chronic cardiovascular adaptive response. In this chapter, we will briefly describe the most common kinds of animal models of acute and chronic cardiovascular exercises that are currently being conducted and are likely to be chosen in the near future. Strengths and weakness of animal-based cardiac exercise modalities are also discussed.

Keywords Exercise • Animal models • Physiological mechanism

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1 Introduction

Exercise affects virtually every organ and contributes to many cardiovascular health benefits. Current concept of exercise was further extended as an active therapy which can prevent or enhance various chronic pathological conditions including cardiovascular diseases. Thus researchers are trying to understand the benefits of exercise in the cardiovascular system, as well as the underlying molecular biological mechanisms behind it. Literature review shows exercise as a simple and low-cost lifestyle intervention and has shown its feasibility, desirability, and practicality. However, there is a need of using different animals to study exercise due to concerns such as research ethics, duration, time and technical difficulties regarding human studies [1, 2].

Numerous exercise research protocols have been established and used appropriate animal subjects to study the impacts on cardiovascular health and diseases [3–8]. Proper exercise models using animals are necessary to study cardiophysiological responses and to develop strategy to recover from cardiovascular abnormalities [4, 9, 10]. The study of exercise physiology is concerned with how the body adapts physiologically to acute stress of exercise and the chronic stress of physical training [3]. Any specialized exercise models developed for testing acute and chronic exercise effects on specific physiological issues should well understand how the animal model-recorded factors are reflected in the human physiological outcomes [3, 8]. For example, humanized animal exercise models providing insight into the understanding of exercise and exercise physiology were summarized in another study [11].

It is clear that animal models are an essential tool in cardiovascular researches where numerous cardiovascular functions and therapeutic targets can be studied. The goal of animal cardiac exercise research is to improve on how we view human health and disease in cardiovascular system and build on this improved understanding to further advance clinical outcomes [6]. In general, animals used in cardiovascular exercise studies are enormously varied from species to species, ranging from small rodent animals (e.g. mice, rats) to large animals (e.g. rabbits, canine, goats, sheep, pigs, horses) [5, 12–16]. It was previously reported that exercise-induced autonomic regulation was changed in a species-dependent manner [16]. Even within a species, several indexes (e.g., animal's size, gender, age) could be considered in order to select suitable animals to achieve the research goals [3]. In animal models, exercise can be either voluntary (e.g. housing animals with a running wheel) or forced (e.g. placing animals on a treadmill for a certain period of time). The determinants for achieving expected beneficial effects of exercise are largely dependent on the designed protocols with chosen animals (species), different types (aerobic, anaerobic), intensities (vigorous-, modulate-, low-intensity), time (morning, evening), and duration (acute, chronic) [16–19]. Animal-based cardiovascular exercise modalities mostly include treadmill running, swimming, voluntary wheel running, usually coupled with a series of other parameters such as varied intensities, times, metabolism, and durations [17, 20–23]. Each chosen experimental protocol should also describe whether respective exercise treatment has produced the expected acute

or chronic cardiovascular adaptive responses. Ultimately the research goal is either to uncover the cardiovascular physiological mechanism of exercise or to uncover how exercise affects cardiovascular health and disease. The latter sections will briefly describe the most common kinds of animal models of acute and chronic cardiovascular exercises that are currently being tested and are likely to be employed in the near future.

2 Criteria for Choosing Animal Cardiac Exercise Modalities

Different criteria may be applied for each exercise modality during the training or conditioning period. These criteria should be reliable and practical without impacting the study's primary aims. On the other hand, performance design and implementation standards are keys to success [5, 7, 15, 24]. In order to optimize exercise protocol, animal experiments designed for assessing the impact of exercise on health and physiology should perfectly address at least several concerns [3].

First, the experimental protocol should exert minimal amount of exercise to produce the expected outcomes (e.g. intensity, duration, frequency) [3]. In reality, many studies have shown that the intensity (low, high), timing (morning, evening), and duration (continuous, discontinuous) of the exercises are the determinants of the physiological responses and outcomes [8, 11, 12, 25–27]. Before initiating any exercise study, animals should be familiarized with the given exercise modalities with a period of adaptation and habituation [3]. This conditioning process is essential to minimize exercise-induced stress responses and injuries. Importantly, researchers must ensure that humane procedures are devoted in either acute or chronic exercise protocols.

Second, exercise study should carefully chose the animal type, which will be selected according to maximal advantages and minimal disadvantages [3]. More care should be also provided in the disease- or disorder-conditioned animals because they are likely unwilling to perform any exercise at all [3, 7].

Third, investigators should also pay attention in selecting exercise type which will best produce the requisite physiological changes with minimal negative consequences brought by stress. For example, while the efficiency and endurance capacity of the heart become more efficient in aerobic exercise, muscle strength and endurance also increase with resistance exercise [11, 28]. Chosen species is also the primary determinant in selecting cardiovascular exercise modalities [1, 6, 7]. These animal exercise modalities, particularly aerobic exercises, include swimming, treadmill running, and voluntary wheel running [10, 17, 20–23, 29, 30].

Fourth, the experimental protocols should be designed to maximize perceived physiological adaptations and to minimize the negative consequences [3]. Protocols should be developed to test the anticipated physiological adaptations to ensure high-quality research and ensure the safety of the research animals (e.g., following the Guide for the Care and Use of Laboratory Animals and ethical guidance concerning about animal welfare) [31].

In addition, careful attention to selection of anesthesia and excision before performing the experiment are essential to the collection of data [3]. Using anesthetic or any drug before can influence several factors in working heart model of rats [32, 33]. Various researches are designed to evaluate the beneficial or detrimental effect of exercise in animals exhibiting specific cardiovascular diseases, such as cardiac hypertrophy, hypertension, ischemic heart disease, and heart failure [1, 2, 10, 15, 17, 20, 24, 34, 35]. As cardiovascular disease models would most likely pose limitations to exercise capabilities or even aggravate an animal's clinical condition, researchers must consider various factors to select the best animal model for their researches. Many of these alternatives are genetically modified models which may offer better choices of achieving experimental goals [31, 34]. However, human and animal hearts are different, depending on the specific animal model used. The advantages and disadvantages of using animal models in studying cardiac contraction deeply underlined [3, 6]. With all animal-based research, a team-based approach to developing performance-based design and implementation standards are needed in order to attain favorable results [3].

Overall, there is no golden standard for which animal model can be used for all cardiovascular researches. Depending on the research goals, animal model needs to be chosen carefully and whether outcomes will be affected and whether these can eventually be adapted for human use [6].

3 Animal Used in Acute and Chronic Cardiac Exercises

3.1 Modalities

As previously mentioned, efficiency and endurance capacity of the heart become more efficient with aerobic exercise and interestingly muscle strength and endurance increase with resistance exercise [11, 28]. The muscle activity performed during exercise has both mechanical and metabolic properties which can differ significantly [28]. Review of previous literatures have shown that aerobic exercise models are most suitable in studying cardiovascular functions [1, 3, 20, 22]. Acute exercise is defined as a single bout of physical activity or exercise, and regular bouts of acute exercise eventually lead to what is termed as chronic exercise. Accordingly, experimental aerobic exercise models in animals use either voluntary or forced activity. Effects of both acute exercise and chronic exercise have been well characterized and reviewed in physiological conditions, and was found to affects human cardiovascular disorders or diseases and animal disease models [36]. A wide range of cardiovascular changes after an exercise from studies in animal models is linked with behavioral effects in human [6, 8, 11, 36]. Normally, physiological changes are described as an acute or chronic activation of the stress response [37, 38]. These stress responses are normal and adaptive facets of body in order to maintain or restore homeostasis to acute exercise. Somehow, the common problem of exercise studies is that chronic or

prolonged stress can significantly affect animal condition and can affect the experimental variables. Furthermore, a significant body of work shows that the range of animal exercise protocols (training type, intensity, time, duration, species, and sizes) has presented a diverse set of findings [1, 3, 18, 24, 25, 39–41]. Considering that acute exercise intensity is a major factor influencing the cardiovascular function (regarding low-, moderate-, and high-intensity) [41], understanding the changes induced by a singular bout of exercise may provide novel insights on how to approach the effects of chronic increases on cardiovascular function [42]. Investigators can also lower stress response activation by maximizing the animal's perceived behavioral control of the exercise situation. In the next section, we will focus on reviewing for aerobic exercise applied in animal cardiovascular researches.

3.2 Aerobic Cardiac Exercise Models

Aerobic exercise models for studying cardiovascular function (arrhythmia, cardiac hypertrophy, coronary artery disease, hypertension, myocardial infarction,) include treadmill running [1, 24, 31, 43–47], wheel running and rotating tract running [1, 3, 48], and swimming [3, 21, 37, 40, 49] (as presented in Fig. 4.1 adapted from

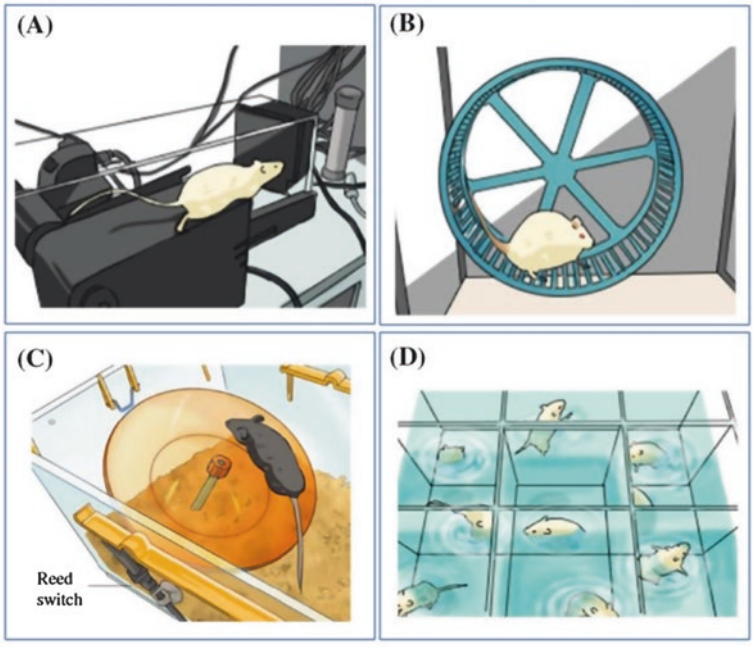

Fig. 4.1 Aerobic animal exercise models (Adapted from published review [11]). (**a**) Treadmill running. (**b**) Elastic wheel running. (**c**) Rotating tract running. (**d**) Swimming

previous review [11]). While treadmill running and swimming are involuntary types of exercise, wheel running is a voluntary exercise. These aerobic exercise models with either voluntary or involuntary are extensively used to investigate the determinants of exercise performance or interventional effects on various cardiovascular pathological conditions, including chronic heart failure [11]. Treadmill running and swimming are widely used as aerobic exercise in small animal rodents and in larger animals. In moderate intensity or duration-controlled aerobic exercises, the duration is performed in the acute or chronic mode of exercise in animals [1, 3] leading to a reduction in blood pressure in hypertension [50], an induction of physiological cardiac hypertrophy, and cardiac remodeling [1, 15, 51, 52].

3.2.1 Treadmill Running Exercise

Treadmill running exercise, an aerobic exercise, is a simple and yet effective modality widely used in cardiovascular researches. In this modality, several animals can be simultaneously trained [3]. Treadmill running can be performed in a continuous manner with a fixed or progressively increasing parameters such as inclination, speed, and duration (minutes to hours) [1, 18, 39, 43]. This exercise can be performed in an interval training model allowing for high-intensity running bouts (successive 4–8-min high-intensity treadmill running bouts at 85–90% of maximal oxygen consumption (VO2max) achieved by running speeds of over 30 m per min on a 25° inclined treadmill during the exercise sessions [22, 42, 43, 51].

Interval running is widely used in studying exercise-induced animal cardiac hypertrophy [1, 34]. This can reduce the impact of the metabolic syndrome and immensity of the effect depending on the intensity [22, 42]. In other exercise researches, the finding outcome suggested that compared to moderate intensity, high-intensity exercise training was more beneficial in reducing cardiovascular risks or heart failures in small rodents (rats, mice) with the metabolic syndrome [22, 42]. This was linked to significantly improved VO2 max, endothelial function, blood pressure, tissue metabolic parameters [22]. Increased mitochondrial density was also reported in study applied this modality [42]. That data documented that a higher training intensity is required to activate mitochondrial biogenesis and cardiac efficiency in the mice heart [42]. Thus, the significance of training intensity is important to trigger metabolic improvements in the myocardium, and it could promise a potential therapeutic for heart failure patients [42]. In fact, not all exercise trainings are equally beneficial, but high intensity training could be a vital therapeutic strategy for treating patients who were once advised to have bed rest [53]. Endurance treadmill training could be a safe and effective non-pharmacological means of maintaining an optimal cardiac autonomic balance, improving cardiac electrical stability, and therefore eliminating risks of sudden cardiac death [5].

Overall, it is clearly accepted that treadmill running is a promising forced model which can be used for aversive stimuli to induce appropriate exercise modality for

research goal, and can be designed with reproducible parameters such as distances and speed. But oftentimes the experimental conditions are stressful and the stimulus to exercise is unpleasant, which may confound outcomes of exercise research goals.

3.2.2 Swimming

Swimming exercise may result in high alternating loads to the cardiovascular system [1, 3, 38, 52]. This exercise can be performed spontaneously with a relative amount of animal and a range of intensities, and it requires a simpler apparatus compared to treadmill running and spontaneous wheel exercise [20, 52].

After a shorter period of familiarization, animals are able to perform exercise with less attention [29]. Swim exercise-induced cardiac hypertrophy model was generated to study the susceptibility to arrhythmias and determine molecular mechanisms underlying exercise-induced cardiac hypertrophy in small rodents (rats, mice) [52, 54]. Interestingly, exercise model using swimming apparatus to control duration, load, and frequency of exercise applied to mice with or without the weight workload attached to the tail of the mouse was designed [52]. The findings demonstrated that duration- and frequency-controlled exercise training similarly induces a significant conditioning response similar with the study done in humans, and the optimal conditioning protocol to induce physiological hypertrophy was 90 minutes, two times a day, 5 days a week for 4 weeks without overload [52]. In the swimming exercise, moderate intensity exercise consists of 1 hour per day and 5 days per week for 8–10 weeks was the optimal protocol [20, 34]. In another study, swimming induced cardiac hypertrophy and hemodynamic changes, but it does not protect the heart against the induction of ventricular fibrillation [49]. Either moderate or high intensity swim training can have an effect on intrinsic calcium current characteristics in rat myocardium [20]. In addition, swim training can prevent changes in acetylcholinesterase and butyrylcholinesterase activities in hypertensive rats exposed to 6 weeks interval swimming training, trained 5 times per week in an adapted swimming system for 60 minutes a day, gradually increasing the workload up to 5% of animal's body weight [29]. In contrast, the susceptibility to ventricular fibrillation was either reduced [54] or unaltered [49] in the isolated, non-ischemic heart of the swimming-trained rats.

Lack of graded workload protocols and the interference of water in the recording equipment can be disadvantageous for swimming exercise. In exercise research, control or sedentary animals are normally placed in shallow water (about 5 cm in depth) at the same temperature and for the same duration as the experimental group, but without a workload to exclude the effect of water [29]. However, there is a need for closer examination on the ways in which stress-induced modulation of behavior in the force swimming test is employed [38].

3.2.3 Wheel Running

In contrast to treadmill and swimming, wheel running is voluntary exercise. Voluntary wheel running is usually adopted to examine chronic exercise in rodent species. It involves the use of running wheels manufactured with stainless steel and plastic (Fig. 4.1b) or the use of an angled rotating running track (Fig. 4.1c) [17]. Animals will spontaneously start running if given access to a freely rotating wheel [9, 48]. This spontaneous exercise can be performed with minimal intervention by the investigators, is less stressful to the animals, and can be performed with varying resistance loads as summarized in a previous review [11]. Since animals can be housed in wheels for long periods of time with minimal disturbance, the wheel running exercise is appropriate for aging studies [55]. Furthermore, wheel running are able to promote physiological cardiac remodeling involving a set of microRNAs, essential in different cellular processes in the regulation of cardiovascular phenotypes [55, 56].

3.3 Animals in Cardiovascular Exercise Studies

Researches regarding cardiovascular physiological and pathophysiological activities are necessary for designing novel treatment options to prolong and improve patients' lives [6]. In fact, exercise is considered as a medicine which can prevent, manage, and regulate numerous cardiac chronic conditions [41]. Regular physical exercise in humans can even reduce cardiovascular risk and reduce death during cardiovascular diseases [41, 57]. However, the beneficial or adverse outcomes of exercise intervention in the treatment of a specific condition should be tested before applying in a clinical setting. Because of research ethics and technical difficulties in humans, animal exercise models are necessary for the future development of exercise mimetics in treatment of cardiovascular abnormalities [11]. In fact, animal models are key aspects of cardiac research where a variety of cardiac pathophysiology and therapeutic targets can be studied [3, 4, 6, 10, 15, 18, 43]. In cardiovascular researches, mouse, rat, rabbit, canine, goat, horse, and sheep are commonly used, having its own strengths and weaknesses. A suitable animal model is still required to study cardiovascular pathophysiology efficiently in order to be reliably used for translational applicability in humans. Despite the preservation and conservation of many aspects of the cardiovascular system, various gaps still exist between animals and humans which must be considered. Differences arising from variations in heart properties and characteristics were described in detail in a previous publication [6].

3.3.1 Small Rodent Animals Used in Cardiovascular Models

Mouse or rat models possess unique properties which make them efficient models for cardiovascular researches. Strengths such as easy handling, short gestation time, and cost effectiveness make them suitable to be used for researches on cardiovascular

physiology and diseases [10]. Pre-clinical trial of cardiovascular pharmacology can possibly be investigated using these small rodents efficiently and with relatively low financial cost.

The most advantageous aspect of utilizing small rodents, such as mice, is the allowance of several *in vivo* cardiac parameters to be measured by applying technological advances such as in making genetic models [31, 43, 58]. Cardiovascular adaptation accompanied exercise training in experimental animal rodent models are dependent on various applied factors such as duration, intensity, time, and frequency [20]. With a motorized treadmill (with speed at 5, 10, 15, and 20 meters per minute on a 10% grade for about 3 minutes at each workload), rats and mice can increase their heart rates by ~40–50% and ~30–40%, respectively [59].

To study the positive effect or effect of exercise training on hearts, investigators used different sample types like *in vivo* hearts [60], isolated hearts [61], cardiac muscle [44], and isolated cardiomyocytes [62]. These studies have also focused on cardiac functional changes induced by exhaustive exercise though echocardiography [60] with changes in left ventricular hemodynamic recorded after an acute bout of exhaustive exercise using pressure-volume analysis [30].

A number of rodent models in exercise-induced cardiac hypertrophy have been made, and a number of endurance exercise trainings effectively induced animal cardiac hypertrophy, such as treadmill running, voluntary wheel running, and swim training [1, 21, 51, 52]. When it comes to inducing physiological hypertrophy, swim training seems to be as effective as treadmill or voluntary wheel running programs [1, 51, 52]. Rat swimming model was used to study functional aspects of exercise-induced hypertrophy in athlete's heart [40]. Authors had demonstrated the potential of assessing left ventricular function in exercise-induced cardiac hypertrophy. Data showed reversible physiological cardiac hypertrophy induced by exercise in rats and characterized cardiac systolic (improved contractility) and diastolic (improved active relaxation and unchanged left ventricular stiffness) functional improvement [40]. Although regular swim training was not associated with increased stress response in chosen rat model, the results from the previous research is limited to young male rats [40]. Rats were also chosen to develop animal model of swimming-trained cardiac hypertrophy to study arrhythmias during an acute period of ischemia [54]. In contrast, swim training of rats either reduced [54] or did not affect [49] the susceptibility to ventricular fibrillation brought about by coronary artery occlusion. Another study noted that endurance training protocols showed improvements as a result of ventricular remodeling, enhanced contraction, and improved Ca^{2+} handling in rats with experiment heart failure [63].

In mice, aerobic exercise may offer beneficial effects for coronary perfusion in the myocardial ischemia area via calcitonin gene-related peptide changes [34]. Mouse cardio-metabolic phenotype models were generated to assess functional cardiovascular fitness via graded maximal exercise testing [43]. Investigators also developed a graded mouse maximal exercise test to improve testing sensitivity and develop translatable parameters to assess functions of cardiovascular fitness in healthy and dysfunctional mice with non-invasive and cost- effective methods [43]. microRNAs were previously found to be necessary players for cardiac growth

induced by exercise, and can serve as protection against pathological cardiac remodeling in mice. miR-222 expression were resistant to adverse wheel running-induced cardiac remodeling and dysfunction after ischemic injury [55]. Another study found that high intensity aerobic interval training (at 95% of peak heart rate) for about 10 weeks resulted in an increase mitochondrial density, suggesting higher intensity in exercise training is required to stimulate mitochondrial biogenesis and cardiac efficiency in the mice heart [42]. This study proved the importance of training intensity for provoking metabolic improvements in the myocardium, thus leading to therapeutic potential of high intensity aerobic interval training for heart failure patients [42]. In addition, gender differences in cardiac hypertrophy could be a factor, exhibiting significant changes in body weight in response to exercise [17]. Thus, there is still a need of assessing the possible influence of sex, age, or species [40].

Though regular exercise training plays a beneficial function in reducing cardiovascular risk, recent studies have documented elevated biomarker levels consistent with cardiac damage after bouts of prolonged exercise in apparently healthy individuals without cardiovascular disease [64–66]. In rat exercise study, elevated myocardial injury-related markers after prolonged, exhaustive exercises exceeded those of in clinical myocardial infarction [66]. This elevation was accompanied with reactive oxygen species generation-induced oxidative damage in rat myocardium [65]. Since potential limitations of a specific type of exercise and conditions of the experiment can compromise the exercising rats and mice, appropriate exercise testing and prescription must be put in place to aid in the assessment and management of cardiovascular disease [23, 43].

There are many inherent differences between rodent and human hearts, particularly cardiac excitation and contractility, that need to be considered when using small rodents as animal exercise models [6]. These differences can serve as a hindrance in the clinical translation of rodent studies to humans [3, 6].

3.3.2 Rabbits

Rabbits are advantageous for research purposes both in cardiovascular health and disease [2]. Previous finding used rabbits documented that exercise training can increase rabbit heart rates from 71 to 112% during peak exercise [67]. Long term exercise modalities was applied for the rabbits in low-speed flat treadmill at a speed of 18–20 meters per minute for 40–60 minutes per day to study hypertension and cardiac heart failure [68, 69]. The research described that in rabbit heart failure model, exercise training evokes an antioxidative effect [69], suggesting its possibility as a model to be used in studying myocardial effects of endurance training [70]. Moreover, endurance training is known to increase cardiac performance and decrease resting heart rate during exercise. The less steep slope of end systolic pressure length relations acquired by occlusion of the descending aorta in the trained rabbits might indicate a structural myocardial remodeling and increased contractile reserve [1, 70].

A greater functional similarity of rabbit myocardium to humans compared to small rodents make them a closer representative of the human heart. In addition,

though other large species of animals such as canine, pigs, and sheep are more similar to the human heart, the cost for managing rabbits is significantly much lower [3]. Despite a close similarity to humans [71], the differences between rabbit and human myocardium result in differential effects in a particular study or therapeutic intervention. Overall, rabbits are practical and efficient as a model for cardiovascular studies.

3.3.3 Canine Models

Canine and human hearts have similar characteristics both at organ and cellular levels. As previously summarized, canine heart rate, body weight, and heart weight which are more similar to humans than smaller models such as mice, rats, and rabbits [3, 6]. Importantly, changes in the heart rates and other hemodynamic parameters are similar between canines and humans [3]. The shape of the force-frequency relationship is a lot closer to humans compared to mice, rats, and rabbits [5, 14, 15]. These characteristics make both myocardial models react similarly in response to exercise [6]. Typically, canines can significantly increase its heart rate of approximately 96–136% during maximal exercise [72, 73].

Moreover, canines are commonly used for studies utilizing exercise in cardiovascular abnormalities, including ischemic heart disease [16, 36]. Endurance exercise training can enhance cardiac electrical stability in subjects at higher risk for sudden cardiac death [5, 16, 74]. Canines were also used for sudden death to study the effects of daily training on cardiac regulation and remodeling. In dogs, daily exercise affects autonomic control of the heart and prevents ventricular filtration induced by acute myocardial ischemia [19]. Endurance exercise training (treadmill running) interestingly is the most effective antiarrhythmic therapy in the canine model of sudden death, the effect of which was conferred by the prevention of ventricular fibrillation after endurance training program [5, 74]. The amelioration of treadmill exercise-induced myocardial ischemia was brought about by the enhancement of coronary vasodilator reserve in dogs [72]. During resting state, coronary vasodilator reserve occurs even in the presence of myocardial ischemia [72]. Another study using calcium-entry blocker diltiazem in conscious dogs improved both regional myocardial flow and function during exercise, leading to a faster recovery of regional myocardial dysfunction in a chronic coronary stenosis model [73]. It also affects neuroendocrine transmitters partaking in autonomic regulation and signaling of canine heart [16]. The exercise-induced change in autonomic tone to the heart was different among species [16]. Overall, even considering the high cost disadvantage, the canine myocardium serves as an appropriate model of human heart.

3.3.4 Swine

Many researches have used swine due to its similarity with excitation-contraction coupling with human myocardium [13, 16, 75–77].

In dynamic exercise training, heart rate ranged from 62 ± 4 (beats per minute, minimum) to 254 ± 9 (beats per minute, maximum); while in untrained group, this value ranged between 91 ± 13 and 273 ± 6 (beats per minute) [77]. Swine can increase their heart rates from approximately 128–219% during exercise which can be attributed to a large heart rate [13, 76]; this value is very similar to the 140–170% which are available in humans [78]. Chronic exercise affects swine cardiac catecholamines and enkephalins, suggesting responsiveness to autonomic control and its capability to alter cardiac function [16]. Another exercise research used mini swines (pigs) as study subjects. In this study, down-regulation of ß- adrenergic receptor was linked with training-associated decreases in heart rate [77]. In another research, swine were employed to test for the effects of post-infarction mitral regurgitation during surgical repair [79] revealing that chronic exercise suppressed ß-adrenergic receptors in the right atrium and is related with reduced chronotropic responses to exercise and isoproterenol stimulation [77]. Pigs have been well characterized as an appropriate model for the study of coronary physiology. Pigs have been utilized as models of myocardial ischemia and myocardial infarction during graduated treadmill exercise training and increased oxygen demand [80]. These animals are also the best subjects for investigating the coronary collateral circulation and exercise physiology and pathophysiology [75, 80]. Taken together, the swine or pig has proven its value for pre-clinical research due to its similarities with the human cardiovascular system, and its characteristically large heart and body weight.

3.3.5 Sheep

Similarly with other large animals, the sheep share numerous similarities with humans which makes it a good pre-clinical model to study cardiovascular diseases [7], including myocardial infarction [81], gradual aortic constriction [82], and tachypacing induced heart failure [4].

Although disadvantageous when it comes to cost and maintenance, sheep-subjected disease models will generally better recapitulate changes in humans and efficacy of novel therapeutic avenues than small animal models [6]. The previous studies [45, 47, 83] demonstrated that maternal exercise (treadmill) decrease uterine blood flow [45] but does not pose a stressful (e.g. hypoxic) event to the fetus as evidenced by blood gases, temperatures, and fetal cardiovascular system assessment [47]. The data proved that relatively constant oxygen delivery to the uterus was managed by means of hemoconcentration adaptation during exercise [45]. Thus redistribution flow towards the placenta after exercise might be a fetal compensatory mechanism [83].

Because of their historical use in cardiovascular research and because of their suitability in investigating pathways involved in pediatric heart-valve calcification, sheep have been widely used as the model of choice for cardiopulmonary bypass procedures [7]. Also, sheep are appropriate subjects to study clinically ischemic mitral regurgitation occurring in myocardial infarction-induced left ventricular remodeling [35]. The evidence showed that annuloplasty provides durable relief

from ischemic mitral regurgitation during an extended follow-up period but does not significantly influence left ventricular remodeling in a clinically relevant ovine model of ischemic mitral regurgitation [35]. Overall, sheep can be a good preclinical animal model for cardiovascular research [7].

3.3.6 Other Animal Exercise Models (Horses, Goats)

As the quality of the overall response to exercise in the horse is very similar to that seen in human and laboratory animals, exercise studies on horses have dramatically increased knowledge of horse physiology and pathophysiology [46, 71, 84]. The responses of heart rate, blood lactate concentration, packed cell volume, and hemoglobin after endurance exercise were evaluated in crossbreed horses [85]. Cardiorespiratory responses to submaximal treadmill exercise were investigated in thoroughbred racehorses [86]. After submaximal training, an increased aerobic power was associated with an increase in maximal cardiac output and stroke volume, a decrease in arteriovenous oxygen difference, and no change in heart rate [86]. In order to better understand the relationship between cardiac structure, mechanics, and overall function adaptation during athletic training, exercise-induced cardiac remodeling in racehorses was designed to study cardiac remodeling in aerobic exercise [36, 87]. A recent review has proposed that cardiac remodeling in response to athletic training in racehorses may provide greater insight into the potential for athletic activity to remodel the heart [36].

Goats, an alternate animal species, performed volitional aerobic exercise for food rewards [3] and was used to study the effects of diet and exercise on fatty lesion of the aorta in a model of high-fat diets [88]. Previous research using pygmy goat suggested that maternal cardiac output response to exercise appears normal, the post-exercise fall in stroke volume, presumed secondary to a reduction in preload, could potentially be harmful to both mother and fetus [89]. Additionally, the elevations in heart rate and stroke volume in pregnancy are not primarily mediated via the autonomic nervous system.

4 Conclusion

Taken together, the positive influence of exercise on cardiovascular health to lifespan has become a topic, and the choice of the animal models is an important determinant of the relative disease model to the human situation [3]. Thus, studies in cardiovascular system should use varied animal models to achieve the scientific goals [6]. The research outcomes should answer whether a certain exercise protocol has produced expected adaptive responses, including detailed concerns related to it.

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Part III
Cardiac Cells Adaptations in Exercise

Chapter 5
Structural, Contractile and Electrophysiological Adaptations of Cardiomyocytes to Chronic Exercise

A. Krzesiak, N. Delpech, S. Sebille, C. Cognard, and A. Chatelier

Abstract Cardiac beneficial effects of chronic exercise is well admitted. These effects mainly studied at the organ and organism integrated levels find their origin in cardiomyocyte adaptation. This chapter try to highlight the main trends of the data related to the different parameters subject to such adaptations. This is addressed through cardiomyocytes size and structure, calcium and contractile properties, and finally electrophysiological alterations induced by training as they transpire from the literature. Despite the clarifications needed to decipher healthy cardiomyocyte remodeling, this overview clearly show that cardiac cell plasticity ensure the cardiac adaptation to exercise training and offers an interesting mean of action to counteract physiological disturbances induced by cardiac pathologies.

Keywords Structure • Contractile • Electrophysiological adaptation • Cardiomyocytes • Exercise

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In spite of decades of research work on effects of physical exercise training on the cardiac tissue, no actual consensus has been obtained on a clear view of such effects on cardiomyocytes.

There are many reasons for this, including the followings: one is the type of animals used for experiments. For evident reasons data from human are absent and the rat, for practical reasons was the only animal used in the long list of exercise effects publications. Furthermore, some authors have used females, others males, each of them having a reason making sense for his choice. The problem is further complicated with the characteristics of the exercise, including the fact the exercise is voluntary (free access to swimming or running vertical wheel) or enforced (treadmill), and the age of the animal on which it was applied, both of them appearing to greatly differ from one paper to another. Finally the source region of cardiomyocytes, mainly the ventricles, also sometimes differed with discrimination between sub-endo and sub-epicardium origin area [1] which have different electrical [2] and mechanical [3] properties, as well as between sinus or ventricle origins.

1 Training Effects on Size, Structure and Proteins Content of Cardiomyocytes

Nevertheless, in the following parts, we will try to highlight the main trends of the data related to cardiomyocytes size and structure, calcium and contractile properties, and finally electrophysiological alterations induced by training as they transpire from the literature. These data will be brought together in the Table 5.1 and tentatively summarized in the Fig. 5.1.

In the following text, chronic exercise and exercise training will be used indifferently and effects of acute exercise is not addressed.

1.1 Size and Structural Aspects

1.1.1 Left Ventricle Weight Increase

A majority of publications begins by reporting data related to the size, therefore the weight, of the ventricles and in particular of the left one. Although this is not directly related to the characteristics of cardiomyocytes, this value is necessary to get an idea of cardiac hypertrophy, which is the most undeniable effect of chronic exercise, and particularly of the type of hypertrophy (physiological or pathological).

While the right ventricle was more rarely measured and seems to be only slightly hypertrophied, the left ventricle weight appeared drastically increased with chronic exercise. Enhanced weight values were reported to range from 7 to 39% depending on characteristics of the applied exercise and on its intensity: 7% [4], 8% [5], 12% [6], 14% [7], 17% [8], 21% [9] or 39% [10]. Whatever was the reached weight amplitude it is clear this mainly explains the whole heart hypertrophy induced by chronic exercise.

Table 5.1 Structural, contractile and electrophysiological effect of exercise training on healthy cardiomyocytes

	Cardiomyocytes content	*Size of cardiomyocytes*	*Transverse Tubules*	*Myosin Heavy Chain*	*Troponin*
Structural	↗ **Mitochondria's number** [9, 12] ↗ **Surface density of S.R.** [9] ↗ **Hyperplasia of the Golgi** [9]	↗ **Cell length** [4-7, 10, 13, 14] = **Cell length** [8] ↗ **Cell width** [7, 8, 14, 15] ↘ **Cell width** [6]	↗ **T-tubules density at center** [14] = **T-tubules spacing** [14]	↗ **α MHC** [19-23] = **α MHC** [24-26] ↗ **β MHC** [26, 27] = **β MHC** [23]	↗ **Troponin** [21] = **Troponin** [31]
	Contractility	*Calcium transients*	*SERCA*	*RyR2*	
Contractile	= **Shortening** [32] ↗ **Shortening** [13] ↘ **Amplitude and velocity** [33] ↗ **Time to peak and time to half relaxation** [7, 10, 15, 34-36]	↘ **Time of ½ decay** [6, 7, 10, 15, 34, 36] ↘ **Time to peak** [6, 7, 10, 15, 34, 36] ↗ **Ca^{2+} transient amplitude** [6, 34, 36]	↗ **SERCA2a** [10, 34, 36] ↗ **PLB Ser16** [6, 36] ↗ **PLB Thr17** [34]	↗ **RyR2 proteins and gene** [44, 66] = **FKBP12.6 gene** [45] ↘ **Frequency of sparks** [45] ↗ **Amplitude of sparks** [45]	
	Action potential	*Potassium channel*	*HCN channel*	*Calcium channel*	*Sodium channel*
Electrophysiological	= **Amplitude** [57] = **Duration** [57] ↗ **Duration** [8] ↘ **Duration** [56, 59]	= **I_{to} density** [57, 59] ↗ **I_{ss} and I_{K1} density** [57] ↗ **Task1 and Kir2.2** [57] ↗ **mRNA SUR2A** [56] ↗ **Kir6.2 and SUR** [58] ↗ **I_{KATP} density** [56]	↘ **mRNA HCN4 and 1 in sinus node** [57] ↘ **I_f density in sinus node** [57]	↘ **mRNA Cav1.2 in sinus node** [53] ↗ **mRNA Cav1.2** [57] ↗ **I_{CaL} amplitude and density** [57] = **I_{CaL} density** [57]	↗ **mRNA SCN5A and SCN1B** [57]

1.1.2 Cardiomyocytes Structures Content Changes Induced by Chronic Exercise

Before the enzymatic dissociation technique was routinely used, some precious data were derived from electron microscopy examination of cardiomyocytes content. Morphometric analysis revealed that training leads to an increase in the volume density of mitochondria, which is explained by an increase of their number [9, 11] as well as a 55% enlargement of the surface density of the sarcoplasmic reticulum (SR) per unit of myofibrils (SR/myofibril ratio) and a pronounced hyperplasia of the Golgi apparatus.

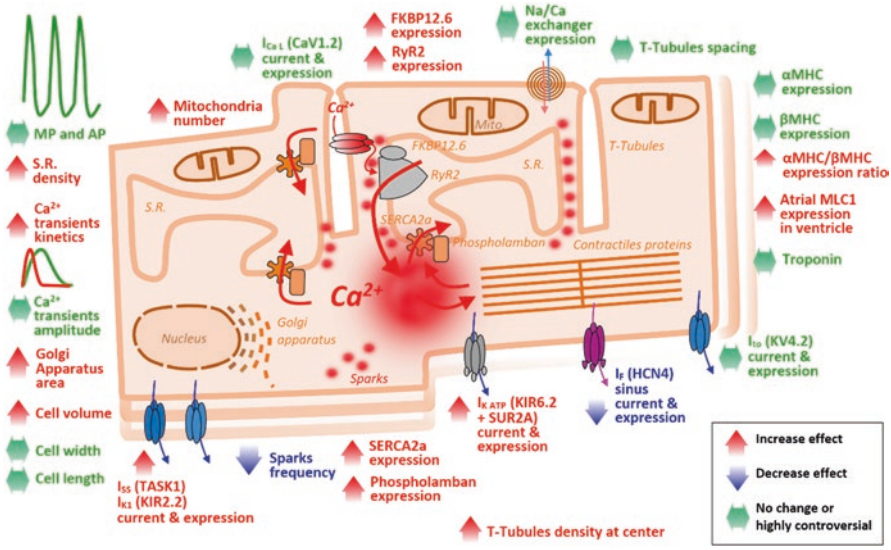

Fig. 5.1 Graphical abstract of training effects on healthy cardiomyocytes. *AP* action potential, *MP* membrane potential, *SR* sarcoplasmic reticulum, *T-Tubules* transverse tubules

Some 35 years later, using a specific scoring technique, Nie and colleagues [12] compared the "injury score" of mitochondria from left ventricles of two groups of control and trained rats in a tentative to highlight some difference but without success, demonstrating that training in healthy animals is not deleterious.

1.1.3 Cardiomyocytes Hypertrophy: Resulting Effects of Exercise on Length, Width and Depth

With the increased use of the enzymatic dissociation technique and the extensive implementation of confocal microscopy it became easier to address the length, width, depth and the resulting volume of isolated cardiomyocytes.

Cell length was generally found to be increased, ranging from 5% to 20%: 5% [4, 13], 6% [5, 6], 10% [14], 13% [10], 20% [7]. It should be noted this length increase is reversible. In the latter paper, authors reported a detraining in 2 weeks, in a timing similar to the left ventricle weight which returned to 2% (over 14% increase) in 2–4 weeks of detraining.

With regards to regional differences it could be noted that cardiomyocytes originated from sub-endocardium wall left ventricle displayed a greater effect of training than the one from sub-epicardium wall: a 20% cellular hypertrophy (measured as cell volume) was seen in endocardium cardiomyocytes while the size increase was no significant in epicardium cardiomyocytes [1]. Similar results were obtained [8] in experiments where the depth was also measured or calculated.

Nevertheless results related to the increase of cardiomyocytes length is controversial as these authors observed no differences in cardiomyocytes length whatever was the regional origin (endo- or epicardium) or sedentary or trained rats [8].

In this paper and others [7, 14, 15] the main observed effect of training is focused on the width (and by implication on depth) of cardiomyocytes with a greatest hypertrophy effect in endocardium wall region [8]. To further complicate the situation, other data [6] indicated a slight reduction of the cardiomyocytes width (−2%) under training.

1.1.4 Effects Dependence on Training Intensity

Clinical and epidemiological studies suggest that beneficial effects of regular exercise depends on intensity or amount of work performed (see for example [16]). So the group of Wisloff [15] have compared the effects of moderate versus high intensity exercise on different parameters in rats running on treadmill. In these experiments only high intensity type of chronic exercise was able to increase (4%) the left ventricle weight. Regarding the cardiomyocytes length the two types of intensity of training was able to enlarge the cells length but with a prominent effect obtained with the high intensity type (14%) compared to the moderate intensity training (5%). The same trend was observed for the cardiomyocytes width (as well as by implication for volume). By contrast, Wang and colleagues [17] also reported effects of both moderate and high intensity trainings on length and width of cardiomyocytes but without significant differences between the two modes.

1.1.5 T-Tubules: A Minimal Change

One another morphologic parameter that could be addressed was the degree of development and shape of T-Tubules network. Transverse tubules, which are invaginations of the plasma membrane in close relation with the sarcoplasmic reticulum membrane, allows the membrane depolarization and calcium entry uniformly across to the whole cell and initiate coordinate contraction of the cardiomyocyte (see [18]). Alteration or changes of these structures involved in such an important mechanism would have certainly drastic impact on the physiological function of these cells.

Kemi and colleagues [14] studied, through Di-8-ANEPPS labelling, two parameters related to T-tubules membrane system: T-tubules density and T-tubules spacing. Except a slight increase of the T-tubules density at the center (x-axis) of cardiomyocytes, no difference in T-tubules density or spacing could be detected between sedentary and trained animals.

1.2 Training Effects on Contractile Proteins Content

The contractile proteins, like myosin and actin present inside the cardiomyocytes play a key role in the contraction mechanism. Indeed, they are responsible for contraction and relaxation of the cell following the displacement of tropomyosin by troponin in the presence of calcium. Given the improvement in contractility observed following physical training, numerous studies have emerged in order to bring out a possible link between the expression of contractile proteins and the effects of chronic exercise.

1.2.1 Myosin Heavy Chain Protein: Increase in the α/βMHC Ratio

Myosin Heavy Chain protein (MHC), is the major contractile protein in the heart. The vertebrate myocardium presents two isoforms of these protein, α-MHC and β-MHC. The latter is predominant in the human heart but the first is thought to predominate in adult heart rat. These two proteins have an important role in the contractile properties of cardiomyocytes. Changes in either of the α- or β-MHC proteins can be directly linked with cardiac contractility properties change.

Effects of exercise training at the level of expression of these two contractile proteins diverge according to the studies. As previously noted, the observed differences may be due to several training-related parameters such as type of exercise and duration or study model such as age and sex of the animals and other parameters.

A number of studies using swimming training protocol, in healthy rats, have suggested that exercise training induces an increase in α-MHC expression [19, 20]. A high intensity swimming training also allows to increase α-MHC expression by 1.2 fold and the synthesis of the protein by 8.5 fold compared to the control group [21]. Others studies showed an increased in α-MHC isoform expression in rats trained by running, during 10 weeks, ranging from 30 to 75% [22, 23]. Moreover, in the latter paper, authors reported a 75% increase in the α-MHC expression of the gene and a 60% increase of the protein expression following only 1 weeks of running exercises. All these studies suggest that adaptations of contractile elements like MHC proteins seems to be an early event and sustained during cardiac growth as training continued. Nevertheless, some studies, using running as a training protocol during 11 weeks [24, 25] or resistance exercises during 5 weeks [26], have found no evidence for a change in α-MHC expression. Results related to the level of α-MHC expression continue to be controversial.

On another side, a number of studies using treadmill or resistance training, showed a decrease in β-MHC expression. A halving of the expression of this protein has been described following chronic aerobic exercise in rat myocytes isolated from the endocardium and epicardium [27] or after 5 weeks of resistance training [26]. However, another study using running as a training modality, suggested no change in the gene or protein expression of β-MHC [23].

Even if some results remained divergent in absolute values, the increase of α-MHC expression and the decrease of β-MHC expression observed in some of

these studies are consistent with data from Soci and colleagues showing an increase in the ratio α/βMHC during long term swimming training at low intensity [28].

Otherwise, myosin also has a second chain called myosin light chain (MLC). A study using a treadmill training program showed an increase in the expression of atrial myosin light chain 1 (aMLC-1) in the trained ventricular tissue of healthy rats [29]. Same authors also suggested that the increase is greater in myocytes originating from the sub-endocardial region compared to the cardiomyocytes isolated from the sub-epicardium [30].

1.2.2 Troponin

Troponin is a protein complex that plays a predominant role in the contraction of the heart muscle and has three distinct subunits. The Troponin C which is responsible for binding to calcium, the troponin I allowing the inhibition of actin binding myosin and the troponin T binding to tropomyosin.

Despite the few studies on this protein, the effect of physical training on the level of troponin expression appears to be divergent between rats and humans. A study using swimming training protocol, in healthy rats, has suggested that exercise training induces an increase in the expression of cardiac troponin and this increase is greater if the training protocol is carried out at high intensity [21]. By contrast, a study in older men and women subjects, following 12 or 24 weeks of resistance training, suggests no difference in cardiac troponin T and I expressions with values of 6,4 and 4,1 ng/L before the protocol and 6,1 and 3,8 ng/L respectively for troponin T and I, after a 24 weeks training [31]. The observed differences between species can be due either to the type of training or to the model of study used.

2 Calcium Homeostasis and Contractile Under Exercise Training

2.1 Contractility and Intracellular Ca^{2+} Transients

The initial results related to the effects of exercise training on cardiomyocytes contractile function also were non-consensual. Laughlin and colleagues [32] first studied the effects of endurance training on cardiomyocytes function. After 16 weeks of progressive treadmill training, the shortening characteristics during a 0.2 Hz electrical stimulation of ventricular myocytes of trained male rats did not differ from sedentary ones. Moore and colleagues [13] observed an exercise training induced increase of shortening on rat cardiomyocytes stimulated at 0.07 Hz. Zhang and colleagues [33] demonstrated a decrease in maximal shortenings amplitude and velocity in ventricular myocytes of sprint trained-male rats during 8 weeks. Discrepancies of the results could be explained by different experimental conditions, e.g. different

type of exercise training, temperature, cell isolation protocol, regional cardiac differences or stimulation frequency. However, differences seem to be lesser in studies using aerobic treadmill controlled-training. Thereby, series of experiments using mouse and rat models highlighted that endurance exercise training improves cardiomyocytes shortening, time to peak of contraction and time to half relaxation [6, 7, 10, 15, 34, 35]. Interestingly, the groups of Kemi [7] and of Carneiro-Junior [36] demonstrated in rat ventricular myocytes that improvement of cardiomyocytes contractility induced by 10 or 8 weeks of endurance exercise training respectively reversed after 4 weeks of detraining. This demonstrates that aerobic exercise training induces adaptations of cardiomyocytes including their contractile characteristics.

At each cardiac cycle, a transient rise in intracellular Ca^{2+} occurs that will trigger contraction (systole). Immediately thereafter, the decay of intracellular Ca^{2+} will cause relaxation (diastole) of cardiomyocytes. Several studies have examined the effects of exercise training on both cardiomyocytes shortening, and intracellular Ca^{2+} transients. Some authors reported a decrease in both systolic and diastolic intracellular Ca^{2+} in cardiomyocytes of exercise trained rats [10, 13, 37]. These results show that improvement of cardiomyocytes shortening by exercise training is not necessarily associated with an increase in systolic intracellular Ca^{2+}. It can be also explained by a greater Ca^{2+} sensibility of myofilaments [10]. Moreover, if other studies showed no effect of physical training on both systolic and diastolic intracellular Ca^{2+}, reductions of time to peak and half-time of decay of intracellular Ca^{2+} transients [7, 15] reported in these works confirm the beneficial effect of training. Indeed, improvement of intracellular Ca^{2+} transients kinetics, also observed associated with increase in systolic intracellular Ca^{2+} in studies by the groups of Kemi [34] and Carneiro-Junior [6], reflect the improvement of Ca^{2+} cycling induced by exercise training.

2.2 Calcium Homeostasis

2.2.1 Ca^{2+} Cycling

Cardiomyocytes contraction results from massive Ca^{2+} release from sarcoplasmic reticulum (SR), actin-myosin-Ca2+ binding interactions and eventually sarcomere shortening. The signal for actin-myosin interaction is the binding of intracellular free Ca^{2+} on troponin C. Intracellular free Ca^{2+} is increased due to the known process Ca^{2+} – induced Ca^{2+} release. The latter takes place as follow: 1/depolarization of both sarcolemma and T-tubules membrane activates L-type Ca^{2+} channels current which allows entry of a small quantity of Ca^{2+} by L-type Ca^{2+} channel and by Na^{+}/Ca^{2+} exchanger (NCX) which works in the so-called reverse mode. 2/Free Ca^{2+} stimulates the ryanodine receptor (RyR2) localized on membrane of SR. 3/A rapid transient of Ca^{2+}- release via RyR2 produces the trigger signal for cardiomyocytes contraction. During the relaxation, Ca^{2+} is removed from the cytosol by both the

cardiac isoform of SR Ca^{2+} ATPase (SERCA2a) and sarcolemmal extrusion via NCX (in forward mode) and to a lesser extent via the sarcolemmal Ca-ATPase. In rats and mice SERCA2a accounts for about 90% of cytoplasmic Ca^{2+} removal [38]. SERCA2a activity is regulated by phospholamban (PLB). Non-phosphorylated PLB is linked to SERCA2a and inhibits its activity; phosphorylation of PLB removes it from SERCA2a which in turn is activated. Both cAMP-dependent protein kinase (PKA) and Ca^{2+}/calmodulin-dependent kinase II (CaMKII) phosphorylate PLB respectively at serine Ser-16 and at threonine Thr-17 [39].

2.2.2 Effect of Exercise Training on Ca^{2+} Cycling

Kinetics of both Ca^{2+} transients signal and contraction-relaxation signal are similar suggesting a close relationship, indicating that the change in contraction-relaxation rate induced by exercise training could originate from changes in rate of Ca^{2+} cycling [40]. Many stages of Ca^{2+} cycling have been identified as potential targets of physical training.

Several studies have shown an increase in SERCA2a protein expression after aerobic training of mice and rats [6, 10, 34, 36, 40, 41]. Thus, an increased Ca^{2+}-uptake capacity of the SR due to an increased SERCA2a expression could account for improvement of contractile cardiomyocytes function. Depending on the studies, the increase of SERCA2a expression originates from phosphorylation of PLB on Thr-17 by CaMKII or on Ser-16 by PKA. Kemi and colleagues [34] observed on cardiomyocytes trained mice an increase of SERCA2a expression associated with an increase of phosphorylation of PLB at the Thr-17 as well as with an increase of CaMKII expression. Kaurstad and colleagues [35] showed that chronic CaMKII inhibition blunts the cardiac contractile response to exercise training of mice cardiomyocytes. On the contrary, Carneiro-Junior and colleagues [6, 36] reported an enhancement of SERCA2a expression and phosphorylation of PLB at the Ser-16 by PKA in cardiomyocytes of trained rats.

Some studies explored the effect of exercise training on the second system of cytosolic Ca^{2+} extrusion, the Na/Ca exchanger (NCX). No changes in NCX levels have been observed by Wisloff and co-workers [10] and Laughlin and associated [42] respectively on rat and porcine models. On the contrary, Tibbits and coworkers [43] demonstrated increase in the affinity of the exchanger for Ca^{2+} in rats.

Another target of exercise training-induced improvement of calcium cycling could be RyR2. Shao and colleagues [44] and Carneiro-Junior and colleagues [45] have highlighted an increase in RyR2 protein content and RyR2 gene expression induced by aerobic training respectively in rat cardiomyocytes. Clusters of RyR2 constitute calcium release units (CRUs) [46]. The release of Ca^{2+} during excitation-contraction coupling is determined by CRUs and is influenced by tight junction protein-protein interactions with FKBP12.6. FKBP12.6 is anchored in RyR2, forming a complex that stabilizes and regulates the closed state of the RyR2 preventing intracellular Ca^{2+} leak [47]. Carneiro-Junior and his group [45] in the same work highlighted no effect of exercise training in FKBP12.6 gene expression. Calcium

sparks are local increase of Ca^{2+} elicited by synchronized opening of RyR2, at rest, without excitation by L-type Ca^{2+} channels current. An excessive increase in spontaneous Ca^{2+} sparks could generate Ca^{2+} waves producing abnormal cardiac electrical activity and reflected an impaired working of RyR2. Shao and colleagues [44] and Carneiro-Junior and co-workers [45] reported both a decrease in frequency and an increase in amplitude of spontaneous Ca^{2+} sparks in ventricular isolated myocytes after moderate intensity continuous training of rats. Exercise training seems to promote the closed state of RyR2 which is participating to normal working of CRUs and is essential to local control of Ca^{2+} release during excitation-contraction coupling.

3 Electrophysiological Remodeling Under Exercise Trainings

In addition to adaptation of calcium homeostasis and structural remodeling, training induced physiological hypertrophy could also impact cardiac electrical activity [48, 49]. It is therefore important to understand the cellular and molecular determinants that underlie these electrophysiological remodeling.

3.1 Electrophysiological Effects in Sinus

Chronic exercise is well known to induce sinus bradycardia, characterized by a decrease in resting heart rate below 60 bpm [50, 51]. Whereas bradycardia is widely attributed to autonomous nervous system adaptation to chronic exercise, experiments that block autonomous pathways or that use denervated sinus node still observed a decrease in resting heart rate [52, 53]. This suggest that the sinus node, the cardiac pacemaker, is also impacted by exercise. Many ion channels participate to the action potential of nodal cells. Amongst them, HCN channels and particularly HCN4 support the funny current (I_f) current that control, at least in part, the pacemaker activity [54]. To date, only one study investigated electrophysiological remodeling of nodal cells using trained rat and mice compared to sedentary animals [53]. Interestingly, D'Souza and colleagues [53] reported that chronic exercise induces a decrease in HCN4 channels. This decrease was observed at the mRNA, protein and functional levels through a decrease in I_f current density in freshly isolated nodal cells of trained animal compared to the control group. Furthermore, they highlighted the correlation between the decrease in heart rate or in Vo_{2max} and the decrease of HCN4 mRNA. These results were confirmed *in vivo* using Ivabradine, a specific blocker of I_f [55] that produced a less important reduction in heart rate for trained animals. Interestingly, the bradycardic effect of chronic exercise was reversed when mice were detrained for 2 weeks and this was associated to a marked increase in HCN4 mRNA. To date, this is the only study in our knowledge that investigated the bradycardic effect of chronic exercise on sinus

node electrophysiological remodeling. These authors also observed changes in transcripts of ion channels that participate in cardiac pacemaker activity such as T and L-type calcium channels. Further studies would therefore be exciting to decipher this electrophysiological remodeling.

3.2 Electrophysiological Effects in Ventricle

Chronic exercise induces cardiac physiological hypertrophy that is observed in ventricular tissue and particularly in left ventricle. As a consequence, different studies has focused their investigations on the impact of chronic exercise on ventricular cardiomyocytes electrophysiology. During the plateau of the action potential, a fine balance exists between inward calcium and sodium currents and outward potassium currents. This equilibrium controls the action potential duration (APD) and is therefore crucial for the excitation/contraction coupling. To date, further studies have investigated the impact of chronic exercise on different potassium outward currents remodeling and APD [56–59].

ATP sensitive potassium channels (K_{ATP}) are energy sensing channels that are activated depending on the ATP/ADP ratio [60, 61]. They allow to connect metabolic changes to an outward repolarizing potassium current that decrease the APD [61]. This characteristic is essential to ensure cardiac acute adaptation to energy demand increase during exercise. This occurs through APD shortening during heart rate acceleration that balance cardiac contraction/relaxation function [62, 63]. Zingmann and co-workers [56] have observed that the decrease in APD associated to rhythm elevation was enhanced in trained mice. This was the consequence of an increased K_{ATP} current density without effects on gating properties or ATP sensitivity of the channels. Experiments testing the expression of Kir6.2 and SUR2A, respectively the channel pore subunit and the associated sulfonylurea receptor subunit, indicate a 30–50% increase in both proteins whereas only SUR2A transcripts increased. A rise in Kir6.2 and SUR protein level (60–75%) were also observed in trained rats compared to sedentary animals [58].

Physiological hypertrophy associated to exercise does not necessarily lead to cardiac electrophysiological disorders as observed in pathologic hypertrophy. Indeed, cardiac pathological hypertrophy is characterized by an increase in myocytes size associated to a decreased repolarizing potassium current density and an increase in APD [64, 65]. In contrast, using trained mice or a mouse model of physiological cardiac hypertrophy through PI3Kα expression, a study revealed that left ventricular cardiomyocytes doesn't display difference in resting membrane potential, action potential amplitude or APD [57]. Trained and PI3Kα animals presented an increase in left ventricular potassium currents mainly attributed to I_{k1}, Ito, I_{kslow} and I_{ss} components that were correlated to an increased level of mRNA (e.g.:Kv4.2, KChiP2, Kv2.1, TASK1, Kir 2.2) and proteins. Interestingly, this rise in potassium current was at least partially compensated by an increase in membrane capacitance. This lead to a less important but still significant 10–20% increase in I_k current density and

particularly for I_{ss} and I_{k1} in both animal models and for I_{kslow} PI3Kα mouse model. I_{to} current density was not impacted by training or in PI3Kα mouse model compared to control. In this study, this effect on repolarizing currents was accompanied by an increase in L-type calcium current density in physiological hypertrophy and transcript analysis revealed an increased level of mRNA for Cav1.3, Cav β2, Cavα2δ1. Other transcripts such as SCN5A and SCN1B coding for sodium channel alpha and beta subunits were also upregulated. Finally, their study revealed that physiological hypertrophy induces increase in repolarizing current without change in action potential properties. A hypothesis is that the increase in calcium current depolarizing current balance the increased potassium current to maintain cardiomyocytes action potential. However, another study using rats revealed that training had no effect on I_{CaL} current density [5]. Moreover, it was reported that trained rat displayed a decreased APD, action potential amplitude and a slower dv/dt with a stable I_{to} potassium current density [59]. This discrepancy clearly indicates that further studies are need. One possibility to explain these differences is the different species (mice versus rats) and training protocol used. An interesting point was raised by the study of Natali and colleagues [8]. Using trained rats, these authors highlighted the importance of the differences in cardiomyocytes electrophysiological properties depending on their tissue localization. They observed differences between the epicardium and the endocardium for APD adaptation to chronic exercise. Training induced an increase in APD in the epicardium and had no effects on the endocardium. This point is important and it could explained discrepancy between studies that do not discriminate between endocardic and epicardic cardiomyocytes. As a consequence, it would be very interesting to compare between these different cardiac locations the exercise induced electrophysiological remodeling.

4 Conclusions

As shown in Fig. 5.1, summarizing the data is very difficult as numerous results remain controversial. It should be noted that such a situation results from at least a fundamental difficulty: in spite of the fact that cardiac hypertrophy under training is a solid knowledge, this could not be true at the level of isolated cells (cardiomyocytes) as other types of cells could contribute to the tissue hypertrophy. Despite the lack of consensus, it has been shown that exercise training induced cardiomyocyte hypertrophy (cell volume) although these changes were not clearly related to specific cell length or width modifications. Exercise training also induces contractile and electrophysiological adaptations of healthy cardiomyocytes. Indeed, it has for example been underlined an increase in the calcium transient kinetics or in the expression of many proteins of the excitation-contraction coupling (SERCA2a, PLB and RyR2) but also a decrease of the sparks frequency. Similarly, increased current amplitude is, at least in part, compensated by the increase in cardiomyocyte volume ensuring the good electrical activity of this remodeled cells. Beside, an increase in repolarizing currents could also enhance the ability of the heart to

support heart rate acceleration by the reduction of the APD. However, number of calcium and electrophysiological remodeling remain controversial. Therefore it is highly recommended to scrutinize each article (Table 5.1 and other feature ones) with a particular attention on how the data are expressed and what were the training protocols.

In conclusion, despite the clarifications needed to decipher healthy cardiomyocyte remodeling evoked by chronic exercise, training beneficial effects are well admitted. Cardiac cell plasticity ensure the cardiac adaptation to exercise training and offers an interesting mean of action to counteract physiological disturbances induced by cardiac pathologies.

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Chapter 6
Formation of New Cardiomyocytes in Exercise

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Abstract Heart failure is a life-threatening disorder associated with the loss of cardiomyocytes. The heart has some endogenous although limited regenerative capacity, thus enhancing cardiac regeneration or stimulating endogenous repair mechanism after cardiac injury is of great interest. The benefits of exercise in heart diseases have been recognized for centuries. Besides the promotion of a favorable cardiac function, exercise is also associated with new cardiomyocytes formation. Exercise may lead to cardiomyocytes renewal from pre-existing cardiomyocytes proliferation or cardiac stem/progenitor cells differentiation. A deep understanding of exercise-induced formation of new cardiomyocytes will enable us to develop novel therapeutics for heart diseases.

Keywords Exercise • Cardiomyocytes • Proliferation • Stem cells • Progenitor cells

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1 Introduction

Heart failure caused by ischemic cardiac diseases is a leading cause of death worldwide [1, 2]. After the onset of coronary artery occlusion, cardiomyocytes undergo apoptosis and necrosis [3]. Myocardial infarction can wipe out one billion myocytes in a few hours [4]. The cardiomyocyte loss during ischemia is also accompanied by severe inflammatory response and local fibroblast activation [5]. As adult mammalian heart has limited potential to regenerate, the self-repair mechanism in ischemic myocardium is largely associated with collagen-rich scar formation [6, 7], which may progressively lead to cardiac fibrosis and eventually develop into ventricular remodeling and heart failure [8, 9]. However, on the other hand, the heart is unable to compensate for cardiomyocyte loss occurring in myocardial ischemia and heart failure. Thus, enhancing cardiac endogenous regenerative capacity might offer novel strategies for heart failure treatment.

Exercise-induced cardiac growth has beneficial effects in the prevention and treatment of cardiac diseases [10–12]. Several studies have reported that exercise might lead to new cardiomyocytes formation by activating resident cardiac stem cells (CSCs) and progenitor cells (CPCs). Exercise has also been associated with enhanced endogenous regenerative capacity by promoting proliferation of pre-existing cardiomyocytes. This chapter will summarize recent findings on exercise-induced formation of new cardiomyocytes and the molecular basis of new cardiomyocytes formation in exercise, which may provide novel therapeutics for heart diseases.

2 Limited Cardiac Regenerative Capacity

The heart has long been recognized as a postmitotic non-regenerating organ [13, 14]. Cardiomyocytes possess the proliferative capacity during fetal life but exit the cell cycle soon after birth in mammals [15]. It has been speculated that the changes to cardiomyocytes during this time period, including conversion of glycolysis to fatty acid metabolism, increase in cell size, and reduction of proliferative capacity, were an evolutionary advance [16–19]. Adult cardiomyocytes have very complex and well developed cytoskeleton, among which hundreds of sarcomeres are responsible for generating sufficient myocyte contractility in mammals [19]. Furthermore, adult mammalian cardiomyocytes are often multinucleated and polyploid, which might prevent mitosis division. Based on these concepts, the adult mammalian heart has long been considered as having no potential to regenerate and cardiomyocytes were only presumed to undergo hypertrophy, senescence, and death after myocardial infarction [20]. However, low rate of apoptosis exists in normal adult heart and is enhanced during ageing [21]. In this regard, cardiomyocyte renewal is speculated to be necessary to compensate for apoptosis-associated cardiomyocytes loss in order to balance the volume and function of heart.

To date, increasing evidence has confirmed that the adult mammalian heart had a certain degree of self-renewal [22–25]. Different strategies were used to measure cardiomyocyte turnover. The 4-OH-tamoxifen-induced labeling of pre-existing cardiomyocytes with green fluorescent protein (GFP) was utilized in double-transgenic MerCreMer-ZEG mice [26]. This genetic fate-mapping strategy showed that the percentage of GFP-positive cardiomyocytes remained unchanged during 1 year of normal ageing, while significantly declined after experimental myocardial infarction or pressure overload [26]. The "dilution" of GFP-positive cardiomyocytes indicates that stem or progenitor cells may refresh adult cardiomyocytes after injury [26]. However, scientists speculated that human may have different requirement for cardiomyocyte renewal due to their much longer life-span than rodents. Based on the high atmospheric level of carbon-14 generated by nuclear bomb tests during the Cold War, convincing evidence was provided for human cardiomyocyte renewal [27]. Through examination of the integration of carbon-14 into DNA of myocardial cells, investigators demonstrated that about 1% of cardiomyocytes were renewed annually at the age of 25, which gradually declined to 0.45% at the age of 75 [27]. Overall, nearly 50% of cardiomyocytes would be renewed during a normal human life span, though whether the new cardiomyocytes were derived from pre-existing cardiomyocytes or cardiac stem cells was unclear [27]. More recently, the multi-isotope imaging mass spectrometry (MIMS) was utilized to study cardiomyocyte turnover, which identified pre-existing cardiomyocytes as the dominant source of cardiomyocyte replacement during normal ageing [28].

3 Potential Cellular Sources of New Cardiomyocytes in the Adult Heart

The concept of very low rate of cardiomyocytes turnover in the adult mammalian heart has generated a broad focus on finding the potential cellular sources of new cardiomyocytes. Evidence has indicated that newly-formed cardiomyocytes may derive from CSCs/CPCs or pre-existing cardiomyocytes [28, 29] [30].

3.1 CSCs and CPCs

The activation and differentiation of stem cells and progenitor cells is essential to regulate tissue homeostasis in most human organs. CSCs, a group of undifferentiated cells which have the ability to self-renew, are originally characterized by cell surface marker c-kit [31]. In general, stem cells settle in niches which constitute the microenvironment to keep their undifferentiated state [32–34]. Once activated, CSCs divide symmetrically or asymmetrically to generate cells committed to new CSCs and differentiate into cardiac cell lineages [35]. Accompanying with further

investigation of CSCs, several additional and distinct CSC classes have been detected such as Sca-1 positive cells, Islet-1 positive cells, side population-Abcg2 positive cells, and progenitors generating cardiospheres [36–40]. The c-kit positive CSCs are multipotent to give rise to cardiac myocytes, smooth muscle cells, and endothelial cells [41]. However, the multipotentiality of Sca-1 positive or Islet-1 positive cells is an open issue to be addressed [42–45]. Compared with CSCs, CPCs are a group of immature but tissue-specific cells that can proliferate and develop into one of the main cardiac cell lineages (myocytes, vascular smooth cells, or endothelial cells) [46]. However, it is difficult to discriminate between CSCs and CPCs, as they may represent different developmental stages of the same cell population and specific markers for CSCs and CPCs are still lacking [47].

Several studies have reported the critical roles of CSCs and CPCs in the turnover of cardiac myocytes during normal life-span [48, 49]. The activation and differentiation of CSCs and CPCs to myocytes has also been shown in ischemic injury and pressure overload [50, 51]. However, other studies have indicated that CSCs and CPCs could not be effectively activated to promote endogenous tissue repair upon myocardial injury [52]. The benefits of CSCs and CPCs might also be due to a paracrine effect [43]. Thus, the relative contribution of resident stem cells to newly-formed cardiomyocytes during ageing or in response to ischemic injury are still debated. To develop novel strategies to enhance the stem cell-derived cardiac myocyte renewal will be of great interest.

3.2 Pre-existing Mature Cardiomyocytes

Although cardiac regeneration has been studied for a long time, little progress has been made in characterizing the mechanisms of mature cardiomyocyte proliferation. Cardiomyocytes undergo DNA synthesis and nuclear mitosis without cytokinesis, which makes a substantial proportion of cardiomyocytes binucleated and withdraw from the cell cycle [53, 54]. It has been proved that cardiomyocyte DNA synthesis activity and cell cycle activity were markedly decreased after birth, however, postnatal proliferation of cardiomyocytes does exist and has been documented in humans and rodents [55].

Investigators have used different methods to determine cardiomyocytes turnover. The 3H–thymidine, a material involved in DNA synthesis, was injected to MHC-nLAC mice to mark the newly generated myocytes, showing a very low rate of myocytes turnover less than 1% per year [25, 56]. The use of genetic fate-mapping with stable isotope labeling and multi-isotope imaging mass spectrometry (MIMS) demonstrated that the origin of newly-formed myocytes mainly derived from division of pre-existing cardiomyocytes both in normal mammalian myocardial homeostasis and after myocardial injury [28]. The turnover rate of cardiomyocytes is approximately 1% per year in adult mice. As the ^{15}N tagging cardiomyocytes were predominantly GFP positive, the cellular origins of new cardiomyocytes were associated with proliferation of pre-existing myocytes instead of cardiac progenitor cells

[28]. Furthermore, the low level of cardiomyocytes proliferation under normal circumstances could be increased in the border zone after myocardial injury [28]. Based on the "mosaic analysis with double markers" mouse model, it was also proved that the differentiated α-myosin heavy chain (α-MHC) expressing cardiomyocytes were the cellular source of postnatal cardiomyogenesis, though cardiomyocyte division is very limited during ageing and even after ischemic cardiac injury [57]. Indeed, a deep understanding of the mechanisms limiting adult cardiomyocyte proliferation may raise the hope of promoting new cardiomyocyte formation after myocardial injury.

4 Exercise Activates Resident Cardiac Stem Cells

Under physiological myocardial ageing, cardiomyocytes undergo telomerase shortening and apoptosis. CSCs and CPCs were supposed, to some extent, to be activated and differentiated to replace the dying cardiomyocytes, and thus maintain the myocardial homeostasis and cardiac function [58]. Equally important, a promotion of endogenous stem cell activation has been proved to protect the heart from cardiac remodeling and dysfunction after myocardial injury [50]. Increasing evidence has shown that exercise was an efficient physiological stimulus to activate and mobilize different types of stem cells, such as cardiac stem cells, skeletal muscle satellite cells, and endothelial progenitor cells [59].

The cardiomyocytes adaptations to exercise result in cardiac growth through both cardiomyocytes hypertrophy and hyperplasia, the former refers to the increase in cell size and the latter refers to the increase in cell number [60]. The potential roles of c-kit positive CSCs were the first being identified in exercise-induced cardiac growth [61]. It was demonstrated that the number of c-kit positive CSCs was significantly increased after intensity-controlled exercise in rats [61]. Interestingly, approximately 80% of the c-kit positive CSCs were either Nkx2.5 positive or Ets-1 positive, indicating that these CSCs were already committed to myocyte or endothelial cell lineage, which probably contributed to the balance between myogenesis and angiogenesis [61]. Exercise also increased the myocardial expression levels of growth factors, such as insulin-like growth factor (IGF-1), transforming growth factor-β1 (TGF-β1), bone morphogenetic protein-10 (BMP-10), neuregulin-1 (NRG-1), and periostin (POSTN), among which IGF-1 and NRG-1 promoted CSC proliferation while BMP-10 and TGF-β1 stimulated CSC differentiation [61]. In addition to c-kit positive stem cells, the Sca-1 positive progenitor cells were also found to be increased in the left ventricle and outflow tract of mice swimming for 3 weeks, accompanied with an upregulation of IGF-1 and hepatocyte growth factor (HGF) [62].

Base on the studies above, exercise-induced activation of resident CSCs and CPCs is presumed to be a physiologic repair or compensation mechanism involved in the cardioprotective response to exercise. However, the mechanisms of stem cell activation and their relative contribution to cardiomyogenesis after cardiac injury need further investigation.

5 Exercise Induces Proliferation of Pre-existing Cardiomyocytes

Increasing data have shown that endurance exercise can induce a proliferative response of adult cardiomyocytes, which is associated with cardioprotective effects. The limited proliferative capacity of cardiomyocytes was proved to be enhanced with endurance swimming [63]. Exercise leads to a reduction in C/EBPβ expression and an increase in CITED4 expression, which is sufficient to promote both hypertrophy and proliferation of primary neonatal rat cardiomyocytes *in vitro* [63] [64]. C/EBPβ knockdown mice develop physiological cardiac hypertrophy and cardiomyocyte proliferation, and are also resistant to pressure overload [63]. However, forced cardiac expression of CITED4 produces physiological cardiac hypertrophy without increasing cardiomyocyte proliferation in adult hearts [64].

The role of microRNAs (miRNAs, miRs), a large group of small non-coding RNAs, in exercise-induced cardiac growth has been extensively studied, and some of them were documented to contribute to exercise-induced cardiomyocyte proliferation. Based on microarrays and qRT-PCRs, miR-222 is found to be significantly upregulated in the heart after swimming and voluntary wheel-running exercise [65]. Importantly, miR-222 promotes both hypertrophy and proliferation of neonatal rat cardiomyocytes *in vitro*, and is necessary for exercise-induced cardiomyocyte hypertrophy and proliferation in adult mice *in vivo* [65]. Additionally, miR-17-3p, a member of miR-17-92 cluster, is identified as a critical regulator of exercise-induced cardiac growth. miR-17-3p contributes to cardiomyocyte hypertrophy and proliferation [66]. Interestingly, overexpression of miR-222 and miR-17-3p are both able to protect the heart from cardiac remodeling and heart failure after ischemia-reperfusion injury [65, 66].

6 Potential Role of Exercise-Induced Cardiomyocyte Renewal in Treating Cardiac Diseases

Exercise-induced cardiac growth is a physiological adaptive response associated with myocyte hypertrophy and renewal and angiogenesis as well [67–69]. Clinical studies have proved the cardioprotective effects of exercise, which is now becoming an effective non-invasive adjuvant therapy for many cardiac diseases [70–72]. Exercise not only reduces cardiac risk factors [73–75], but also significantly reduces cardiovascular events [76, 77]. A study recruiting more than 1000 patients has documented that the more participants exercise, the less they will suffer cardiovascular death [78]. Experts recommend that regular physical activity to patients with heart failure is associated with better functional capacity, lower hospital admissions, and reduced all-cause mortality [79]. Although the cardiovascular benefits of exercise have been well established [80], the relative contribution of exercise-induced cardiomyocyte renewal in it is largely unclear.

After myocardial infarction or pressure overload, a large number of cardiomyocytes undergo apoptosis and necrosis, leading to progressive cardiac remodeling and eventual heart failure. Exercise-induced downregulation of C/EBPβ and subsequent upregulation of CITED4 induces neonatal rat cardiomyocyte proliferation *in vitro* [63]. Interestingly, knockdown of C/EBPβ induces physiological cardiac hypertrophy as well as cardiomyocyte proliferation, and also protects against pathological cardiac remodeling after pressure overload *in vivo* [63]. Besides that, forced expression of miR-222 or miR-17-3p, although not sufficient to recapitulate exercise-induced cardiac growth, has been found to promote neonatal rat cardiomyocyte proliferation *in vitro* and prevent cardiac remodeling and dysfunction after cardiac ischemia-reperfusion injury *in vivo* [65, 66]. These studies suggest that exercise-induced physiological cardiac growth and the contributors may provide novel therapeutic targets for cardiac diseases. However, direct evidence is still lacking for the contribution of exercise-induced cardiomyocyte renewal to cardiac regeneration and repair.

Recently, the intraperitoneal injection of 5-Fluorouracil (5-FU) is performed in mice subjected to swimming exercise and ischemia-reperfusion injury to investigate the role of cardiomyocyte proliferation in exercise-induced cardiac growth and exercise-associated protection against ischemia-reperfusion injury [81]. 5-FU is used to attenuate cell proliferation. Interestingly, although 5-FU significantly reduces exercise-induced cardiomyocyte proliferation, cardiomyocyte hypertrophy still develops, indicating that cardiac cell proliferation is not required for exercise induced cardiac physiological hypertrophy. However, the protective effect of exercise against cardiac ischemia-reperfusion injury is totally abolished with 5-FU, suggesting that cardiac cell proliferation is required for the benefits of exercise [81]. Noteworthy, as 5-FU is not specific to inhibit cardiomyocyte proliferation, the loss of benefits of exercise might also be associated with other cell types, such as resident stem and progenitor cells, endothelial cells, and circulating endothelial progenitor cells [81]. It is highly needed to block cardiomyocytes proliferation specifically to investigate the role of cardiomyocytes proliferation in exercise induced cardiac growth and cardiac protective effects.

7 Challenges in Studying Exercise-Induced Cardiomyocytes Renewal

For decades, the dogma was that cardiomyocytes were terminally differentiated cells and the adult mammalian heart was a non-regenerative organ. The capacity of cardiomyocyte renewal in adult heart has not been assessed until recently. With the development of methodology, the notion of cardiomyocytes renewal has been generally accepted by the public. Two main cellular sources for newly formed cardiomyocytes have been recognized including CSCs/CPCs and pre-existing cardiomyocytes [82]. However, the slow self-renewal rate is unable to replace the huge loss of cardiomyocytes after myocardial injury [83]. CSCs and CPCs based

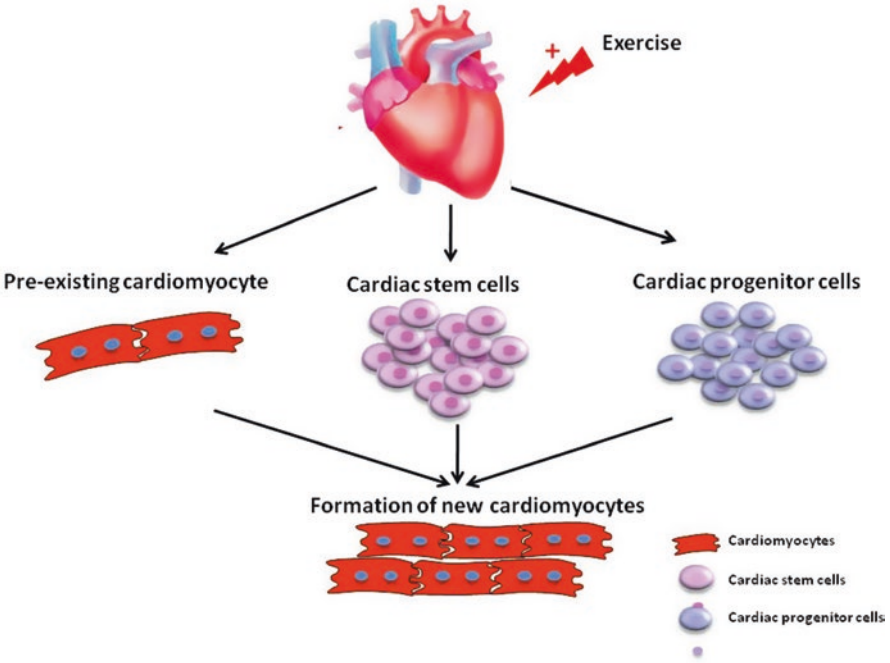

Fig. 6.1 Exercise induces new cardiomyocytes formation through activating cardiac resident stem/progenitor cells or increasing pre-existing cardiomyocytes proliferation

strategies have been largely investigated in the treatment of myocardial ischemia and other cardiac diseases. However, low survival and less attachment of these stem cells after injected into the body may greatly influence the effectiveness of stem cell therapy. Therefore, stimulating endogenous cardiomyocytes proliferation might be an alternative strategy.

Exercise has multiple systemic beneficial effects, including the heart. Recently, exercise has also been demonstrated to promote myocardium self-renewal through activating resident stem and progenitor cells and increasing pre-existing cardiomyocytes proliferation (Fig. 6.1). Although the relative contribution of exercise-induced cardiomyocytes renewal to cardiac repair after myocardial ischemic injury is far from clear, some evidence has been provided that cardiac cell proliferation is necessary for mediating the beneficial effect of exercise against ischemia-reperfusion injury [81].

Finally, the use of exercise as a therapeutic strategy to stimulate endogenous myocardial regeneration may be influenced by multiple variation factors, including patient population, exercise intensity, type, and duration [84]. In such conditions, experts need to define the patient population that benefits mostly from physical therapy, elaborate a personalized exercise program, and establish an effective evaluation method. Importantly, this network will provide the basis for exercise as a useful tool to promote cardiomyocytes proliferation and repair in patients.

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Chapter 7
Physical Exercise Can Spur Beneficial Neoangiogenesis and Microvasculature Remodeling Within the Heart – Our Salvation?

Michal Miko and Ivan Varga

Abstract Economic and social burden of cardiovascular diseases remains enormous and even still rising. There is not enough mass evidence in scientific journals that could describe the course of the process of neoangiogenesis in relatively "healthy" heart after regular endurance exercise. Even though, in this review, we are showing preliminary evidence that this can be one of really cheap and effective preventive means. We are elucidating some of the cellular signaling pathways how exercise could affect neoangiogenesis and ameliorate performance of the heart. Key roles in this process are mechanical forces (mainly increased velocity of blood flow and shear stress) and subsequent rise of angiogenic biological factors (mainly VEGFA).

Keywords Exercise • Neoangiogenesis • Microvasculature remodeling • Heart

1 Introduction

In recent years, we are witnesses of many improvements in treatment of cardiovascular diseases. Nevertheless, economic and social burden remains enormous and even still rising [1]. That is why hopes are directed towards the prevention of such diseases. But effective and cheap cardiovascular preventive treatments remain limited. Concentration on neoangiogenesis within the coronary microvasculature as to one of the possible nodes that can be relatively easily affected by such a simple activity as moderate exercise can be one of these preventive means. It is well known that healthy lifestyle and a regular exercise regimen can help prevent many of

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cardiovascular diseases [2–4]. Mechanisms of maintaining this cardioprotective phenotype are less known and here we want to elucidate some of the physiological processes behind it.

2 Neoangiogenesis in Healthy Heart

First, it is necessary to tell, that information about neoangiogenesis in the physiological terrain of healthy myocardium is scarce. Most of the papers are not dealing with endothelium in myocardium (thus we are pushed only to prudently extrapolate the results for the myocardium) or study the neoangiogenesis in infarction region within the heart wall [5]. Transferring results obtained from skeletal muscle and hypothesize similar results for the heart is therefore speculative and questionable and further works are needed to get a foothold in near future. Other studies e.g [6, 7]. are proving functionality of several pathways with direct positive effect on neoangiogenesis, but they bear no relation to exercise. Recent studies have shown that angiogenesis in coronary microvasculature is caused by endogenous stem cell/progenitor mobilization and participation, and its paracrine effects on endothelial cells function and microvascular distribution. Exercise could mobilize and activate the expression and secretion of endogenous stem cell and angiogenic factors, and affect the cardiac angiogenesis in epigenetics [8].

In this chapter, we are focusing on aerobic, endurance-based exercise (walking, jogging, swimming, skiing, or cycling 3 to 4 times a week or analogous physical activity in animal model [9]) and its role in coronary neoangiogenesis. It is suggested that heavy resistance training in contrast to endurance training does not result in increased capillary density in skeletal muscle [10, 11]. The response of the heart is not dependent on the type of exercise applied, but rather on the duration and intensity at which the exercise is performed [12]. After regular exercise performed for longer period of time, typical functional and even morphological signs of adaptation in vasculature can be monitored. Without these, other morphological signs of adaptation of heart wall as physiological hyperthrophy of cardiomyocytes [13] could be counterproductive. Hyperthrophy (and even hyperplasia [14, 15]), together with neoangiogenesis has a protective effect against cardiovascular diseases [16].

Several cell populations play its role in neoangiogenesis – endothelial cells, endothelial stem cells, pericytes, endocardial cells [17], telocytes [15, 18], partially fibroblasts, vascular smooth muscle cells [19] and cardiomyocytes [20].

Correct structural association between (hypertrophied) cardiomyocyte and newly formed capillaries after exercise is important for maintaining proper function of the myocardium. Each cardiomyocyte possess its own capillary, what can be seen in electron micrographs from scanning electron microscope as well after imunolabeling of endothelial cells around (Figs. 7.1 and 7.2).

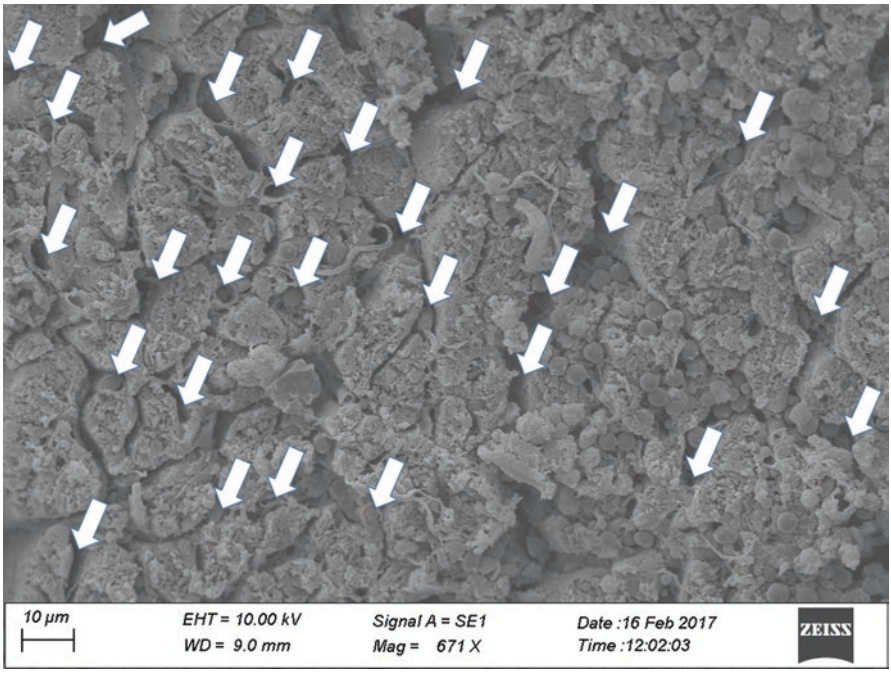

Fig. 7.1 Electron micrograph of a human myocardium from a scanning electron microscope. Rich capillary bed (*arrows*) between cardiomyocytes (Orig. Magn. 671x)

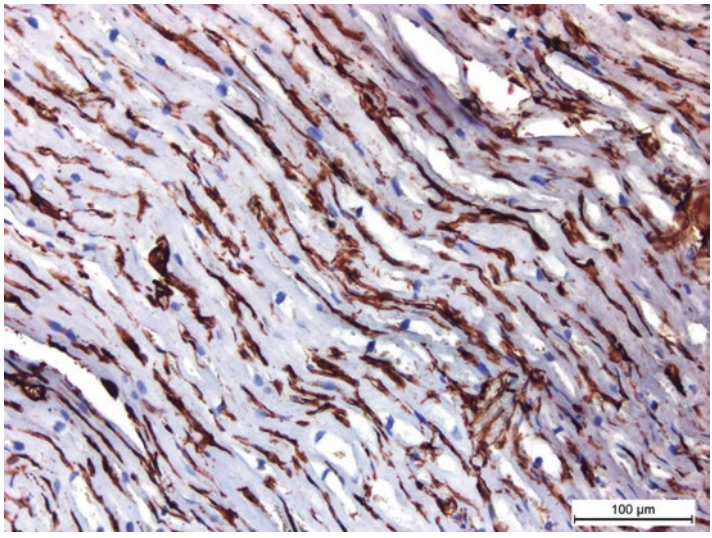

Fig. 7.2 Rich capillary bed of human myocardium. Immunohistochemical labeling of endothelial cells (*brown color*) between cardiomyocytes – each cardiomyocyte possess its "own" capillary (Monoclonal anti-vimentin antibodies, Orig. Magn. 200x)

3 Arteriogenesis

Arteriogenesis (enlargement of diameter of existing vessels) is typically conducted first after exercise. Important function plays increased blood pressure together with shear stress, which act as a mechanical stimulus for endothelial cells [21, 22]. These start to produce cocktail of cytokines as monocyte chemoattractant protein-1 (MCP-1), FGF2, vascular endothelial growth factor (VEGF) or TNF-α that all have positive effect on arteriogenesis [23, 24]. After arteriogenesis comes process of angiogenesis, mainly when arteriogenesis itself is not able to cover demands of tissues. It is an angiogenesis, which is superior to arteriogenesis when talking about effective improvement of exchange properties between blood and tissue.

4 Angiogenesis

There are only few physiological circumstances prompting angiogenesis, notably ovarian cycling or placental development. And exercise is one of them [25]. Essentially, there are two basic pathways for creation of a new mass of functional vessels – sprouting angiogenesis and intussusceptive (non-sprouting) angiogenesis (intussusception). And in the same time, this is the only effective process which is apt to continuously establish improving exchange properties between blood and tissue (Table 7.1).

Sprouting angiogenesis consists of the expansion and remodeling of existing vessels, where the vascular sprouts connect each other to form new vascular loops [26]. Intussusception allows a vast increase in the number of capillaries without a corresponding increase in the number of endothelial cells (no need for activation of endogenous endothelial stem cells). Blood vessels are generated more rapidly in an energetically and metabolically more economic manner, as extensive cell proliferation, basement membrane degradation, and invasion of the surrounding tissue are not required. Mechanical triggers for intussusception are similar as those for arteriogenesis [27].

For those interested, the detailed process of intussusceptive angiogenesis, it is already excellently and comprehensively described elsewhere (e.g. [29]). There is a study from Prior et al. [25] telling that exercise with subsequent muscle contractions

Table 7.1 Sequence of events in sprouting angiogenesis

1. Angiogenic growth factors
2. Activation of receptors on endothelial cells
3. Release of proteases (metalloproteinases like MMP-2) that destroy BM
4. Formation of solid sprouts from migrating endothelial cells
5. Luminization of sprouts

Table 7.2 Sequence of events in intussusceptive angiogenesis [28]

1. Extension of capillary wall into the lumen
2. Splitting of the vessel into two new

create a powerful stimulus for structural remodeling of the vasculature within the random muscle. An increase in flow velocity through a vessel increases shear stress, a major stimulus for enlargement of conduit vessels. This leads to an endothelial-dependent, nitric oxide-dependent enlargement of the vessel. Increased flow within muscle, in the absence of contractions, leads to an enhanced capillarity by intussusceptive angiogenesis [25] and even sprouting angiogenesis [5] (Table 7.2).

5 Neoangiogenesis Promoting Factors Playing Role During Exercise

As a summary of the written, we try to give a closer look to main factors which stay behind neoangiogenesis in the heart wall during exercise. We present them in a logical sequence of steps.

5.1 Mechanical – Hemodynamic Forces

Shear stress acting on endothelial surface induced by elevated radial stress and increased blood flow are main factors which promote vessel caliber changes that tend to restore baseline wall shear stress and that have been reported to be endothelium-dependent [30] and, in the next step, they initiate consequent rise of other angiogenic factors as cytokines. Muscle stretching itself is huge pro-angiogenetic factor in skeletal muscle, however, for cardiac muscle; the role of its increased performance during exercise is a question of further studies.

5.2 Angiogenic Biological Factors

Current thinking is that angiogenesis is mediated by diffusible angiogenic factors and that angiogenic activity is regulated through the balance between stimulatory and inhibitory factors [31]. Recent studies have shown that up-regulation of angiogenic factors occurs in response to increased muscle activity in skeletal muscle [32].

Here we want to discuss some of the chosen biological active molecules incorporated in pathways responsible for neoangiogenesis. We are also mentioning some agents with opposite effect on neoangiogenesis (PEDF) or agents with positive

effects, but generally regarded in negative connotations (chemerin), to show, that investigation is not finished yet and still exist a lot of controversies and that the results sometimes seems quite contradictory.

- **Vascular endothelial growth factor (VEGF):** it is a key and main molecule and it is present in many pathways responsible for neoangiogenesis. The VEGF family comprises in mammals five members: VEGF-A, placenta growth factor (PGF), VEGF-B, VEGF-C and VEGF-D [33]. The latter ones were discovered later than VEGF-A, and, before their discovery, VEGF-A was called just VEGF. A number of VEGF-related proteins encoded by viruses (VEGF-E) and in the venom of some snakes (VEGF-F) have also been discovered. There is evidence based on rat model that exercise training improves aging-induced downregulation of cardiac VEGF angiogenic signaling cascade, thereby contributing to the exercise-induced improvement of angiogenesis [34]. On other hand, a certain degree of restraint is appropriate as another result showed no increased VEGF synthesis after exercise in heart [35]. There are many triggers which can increase the concentration of VEGF that can be produced by several cell types that stimulates angiogenesis. One of the chemical triggers is prostaglandin E1 [PGE(1)]; cardiac myocytes could be a cellular source of PGE(1)-induced VEGF expression [36]. Other trigger can be rise of testosterone levels mediated by exercise. Model of diabetic rat proved that testosterone and exercise can promote neoangiogenesis. The proangiogenesis effect of testosterone and exercise is associated with the enhanced expression of VEGF-A and SDF-1a (stromal cell-derived factor 1) in heart tissue [37]. VEGF-A can act on several receptors – Neuropilin1 (NRP1) is important for coronary neoangiogenesis, it is transmembrane glycoprotein that serves as a receptor for the $VEGF_{165}$ isoform [38]. Biochemical evidence supports a hypothesis of NRP1 function in which VEGF binding induces complex formation between NRP1 and tyrosine kinase receptor VEGF receptor 2 (VEGFR2) to mediate signal transduction of endothelial VEGF signaling [38–40]. VEGF-induced activation of VEGFR2 stimulates endothelial cell proliferation, migration, and differentiation in most cell cultures [41]. From different point of view, VEGF is involved in many pathological conditions as a tumorigenesis and/or atherosclerosis. Bailey with the colleagues demonstrated that after specified exercise, circulating soluble vascular endothelial growth factor receptor-1 (sFlt-1) (an endogenous VEGF inhibitor) is significantly increased in healthy volunteers, which is functionally associated with a transient decrease in circulating free VEGF [42]. From this example is clear that pleiotropic effects of VEGF must be perceived comprehensively and critically. Exercise affects condition of the heart in a positive way, but pathways behind are far more complex as we originally thought.
- **Fibroblast growth factor**: In humans, 22 members of the FGF family have been identified [43] and from these, mainly FGF2, event. FGF1 has neoangiogenic potential. They promote angiogenesis by physical organization of endothelial cells into tube-like structures. Thus, activity of FGFs is stem cells-independent. These multipotent FGFs are true pluripotent or promiscuous growth factors [44].

During exercise, genes for FGF2 receptor are also activated (demonstrated on neutrophils [45]). However, there are signs that concentration of FGF2 is exercise-independent [46, 47]. FGF seem to be effective in initiating neoangiogenesis in hypoxic or ischemic tissues [48].

- **Angiopoietins:** are members of the vascular endothelial growth factor family as the only known growth factors largely specific for vascular endothelium. They act via endothelial cell-specific receptors known as the Ties [49, 50]. There is another group of angiopoietin-like proteins, where at least some of their members (ANGPTL2, ANGPTL8) are found guilty of proinflammatory and proatherogenic actions with effect in propagation of coronary artery disease (cardiovascular disease) [51–53]. We dispose with evidence that levels of these angiopoietins-lige proteins are acutely reduced after physical exercise. A sustained reduction in circulating levels could contribute to the chronic beneficial cardiometabolic effects in patients with coronary artery disease [51].
- **Endothelial NO:** the competency of endothelial NO production is very important in permitting vascular remodeling through arteriogenesis in skeletal muscle arteries. Recognition that chronic endurance exercise with regular bouts of increased laminar flow along the endothelium upregulates endothelial nitric oxide synthase (eNOS) implies that there is an improved responsiveness for vascular remodeling, compared with sedentary individuals [25, 54, 55]. Activity of eNOS in endothelial cells is triggered by pro-angiogenic factors, mainly VEGF. Higher occurrence of NO during physical activity has an antiapoptotic effect that could potentially underlie exercise-related beneficial effects on cardiovascular diseases via increased production and circulating numbers of endothelial stem cells [56].
- **Circulating micro-RNAs (c-miRNAs):** they are short, nonprotein coding RNA molecules. miRNA-126 is vascular endothelium specific and its levels are increased during and shortly after endurance training [57, 58]. miRNA-126, a miRNA located within the egfl7 gene (protein EGFL7 was described as a novel endothelial cell-derived factor involved in the regulation of the spatial arrangement of cells during vascular tube assembly) may be related to exercise-induced cardiac angiogenesis and vascular integrity, by indirect regulation of the VEGF pathway and direct regulation of its targets that converged in an increase in angiogenic pathways, such as MAPK and PI3K/Akt/eNOS [58, 59]. However, experiments in 2015 on human umbilical vein endothelial cells showed exactly the opposite results: transfection with a miR-126 significantly downregulated VEGFA mRNA levels and transfection with a miR-126 inhibitor significantly upregulated VEGFA mRNA levels [60]. Equally, miRNA-129-1 and miRNA-133 act, to our knowledge, as anti-angiogenic factors [61], as well as miRNA-26a [62].
- **Anti-angiogenic factor pigment epithelium-derived factor (PEDF):** human adult cardiac myocytes and fibroblasts constitutively secrete PEDF and inhibit VEGF-induced sprouting. PEDF expression is down-regulated by low oxygen levels [63], thus there is a theory, that PEDF plays a negative feedback-role in regular endurance exercise.

- **Chemerin:** it is an adipokine associated with obesity and the metabolic syndrome, its levels in the bloodstream seems to be highly heritable. It significantly mediates the formation of blood vessels to a similar extent as vascular endothelial growth factor [64].

5.3 Stem Cells Mobilization

In some papers regarding neoangiogenesis, there is no mention about the role of endothelial stem cells. Instead, they are talking about activation of endothelial cells – thus assuming mitotical potential of endothelial cells, both in sprouting and intussusceptive angiogenesis [25]. Exercise training activates circulating, as well as resident tissue-specific cardiac, stem/progenitor cells [65]. Exercise especially induces endothelial progenitor cells to proliferate [56, 66], migrate and differentiate into mature endothelial cells, giving rise to endothelial regeneration and angiogenesis [16]. Endothelial progenitor cells are a subtype of circulating stem cells originally formed in bone marrow, with high proliferative potential, able to differentiate into mature endothelial cells during the neoangiogenesis at trained people [67]. Several physiological and pathophysiological stimuli or drugs modulate endothelial progenitor cell mobilization. Nevertheless, to our knowledge, unequivocal definition of endothelial progenitor cells is lacking [68]. In addition to this direct structural role, endothelial progenitor cells improve neovascularization, also by secreting numerous pro-angiogenic factors able to enhance the proliferation [26, 69].

5.4 Hypoxemia

Findings indicate that lowered oxygen tension may play a role in exercise-induced angiogenesis in skeletal muscle. One of the possible mediating mechanisms could be action of angiogenic factors induced by muscle hypoxia during exercise [47, 70]. Effect of hypoxemia and the question of mere existence of exercise-induced hypoxemia remain unknown and unresponded.

6 Conclusion

As a first thought is important to tell, that questions to which we do not know the answers prevail over those answered and others new are raising as we are trying to understand molecular signaling pathways and mechanisms behind beneficial effect of neoangiogenesis within the heart wall. We were reviewing some studies with similar methodologies and hypotheses, but with completely different outcome (effect of miRNA-126, VEGF levels after exercise, and so on).

And there is not enough mass of evidence in scientific journals that could in more detail describe the course of the process of neoangiogenesis in relatively "healthy" heart. Our work often reflects the formation of new blood vessels in skeletal muscle, which could have a common signaling pathways and morphological manifestations as in both cases we are dealing with cross-striated muscles.

Other source of information are papers dealing with neoangiogenesis in heart suffering from cardiovascular disease. It is known that chronic diseases of the cardiovascular system such as hypertension lead to remodeling of the heart wall, which runs in many ways similar to a physiological adaptation after regular workout.

Nevertheless, one must be extremely cautious and restrained with this approach. That is why presented results of today are at best preliminary and they must be confirmed in studies designed specially to give evidence of link between random (but strictly defined) type of exercise and beneficial neoangiogenesis (or arteriogenesis) within the injury-undisturbed wall of heart. When confirmed, only then one could speculate about the real gains of this process on performance of heart and of the whole organism consequently.

Exercise also induces preconditioning whereby the heart is more resistant to injury even long after the exercise has ceased. The proverbial "triggers" that induce cardioprotective signaling are clearly multi-factorial, and include neural, endocrine, and paracrine factors, as well as autocrine signaling and adaptations that arise from within the heart itself [4].

Important remark must be said to the "unpopularity" of neoangiogenesis. And in most cases, it is indeed well deserved reputation. Neoangiogenesis has been associated with increased aggressiveness of malignant tumors [71, 72] and with diseases as (diabetic) retinopathy [73, 74] or atherosclerotic plaque progression [75, 76]. VEGF as a common molecule of several signaling pathways is involved in many pathological conditions as a tumorigenesis and/or atherosclerosis. Only relatively small step lies between useful angiogenesis in heart and pathological formation of new vessels, mainly if we will have ambitions to interfere into this process affecting one or several molecular pathways. Are we ready to play with fire?

As was said above; there is no question exercise affects condition of the heart in a positive way, but pathways behind are far more complex as we originally thought.

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Chapter 8
The Non-cardiomyocyte Cells of the Heart. Their Possible Roles in Exercise-Induced Cardiac Regeneration and Remodeling

Ivan Varga, Jan Kyselovič, Paulina Galfiova, and Lubos Danisovic

Abstract The non-cardiomyocyte cellular microenvironment of the heart includes diverse types of cells of mesenchymal origin. During development, the majority of these cells derive from the epicardium, while a subset derives from the endothelium/endocardium and neural crest derived mesenchyme. This subset includes cardiac fibroblasts and telocytes, the latter of which are a controversial type of "connecting cell" that support resident cardiac progenitors in the postnatal heart. Smooth muscle cells, pericytes, and endothelial cells are also present, in addition to adipocytes, which accumulate as epicardial adipose connective tissue. Furthermore, the heart harbors many cells of hematopoietic origin, such as mast cells, macrophages, and other immune cell populations. Most of these control immune reactions and inflammation. All of the above-mentioned non-cardiomyocyte cells of the heart contribute to this organ's well-orchestrated physiology. These cells also contribute to regeneration as a result of injury or age, in addition to tissue remodeling triggered by chronic disease or increased physical activity (exercise-induced cardiac growth). These processes in the heart, the most important vital organ in the human body, are not only fascinating from a scientific standpoint, but they are also clinically important. It is well-known that regular exercise can help prevent many cardiovascular diseases. However, the precise mechanisms underpinning myocardial remodeling triggered by physical activity are still unknown. Surprisingly, exercise-induced adaptation mechanisms are often identical or very similar to tissue remodeling caused by pathological conditions, such as hypertension, cardiac hypertrophy, and cardiac

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fibrosis. This review provides a summary of our current knowledge regarding the cardiac cellular microenvironment, focusing on the clinical applications this information to the study of heart remodeling during regular physical exercise.

Keywords Non-cardiomyocyte Cells • Exercise • Regeneration • Remodeling

1 Introduction

Regeneration of tissues damaged by wear and tear or injury, in addition to tissue remodeling as a result of chronic disease or increased physical activity, modify tissue architecture through coordinated cell proliferation, differentiation, dedifferentiation, and apoptosis [1]. These processes in the heart, the most important vital organ in the human body, are not only a fascinating scientific problem, but they are also clinically important. Surprisingly, cardiomyocytes account for only 25–35% of all the cells in the heart. In fact, in the heart, morphologically and functionally distinct cardiac non-myocyte cells (all cells of the heart excluding cardiomyocytes) constitute the majority of cells in this organ [2]. Non-cardiomyocytes are a diverse group of cells and include fibroblasts, telocytes, mast cells, endothelial cells, white blood cells, and other immunologically active cells, such as smooth muscle cells, adipocytes, and pericytes. Exercise is a well-established intervention for the prevention and treatment of cardiovascular diseases. The increase in the size of cardiomyocytes is likely a central mechanism for exercise-induced cardiac growth, but other cardiac cell types also respond to exercise. Therefore, exercise-induced cardiac growth is a complex process that depends upon cross-talk between cardiomyocytes and non-cardiomyocyte cells of the heart [3].

This review summarizes our current understanding of the cardiac cellular microenvironment. It also discusses how this research might be applied to help develop clinical treatments that promote heart regeneration and tissue remodeling. Expanding our knowledge of the diverse non-myocyte cell populations present in the heart is essential for understanding cardiac homeostasis during normal and pathological conditions. Here, we focus on exercise-induced cardiac remodeling.

2 Cardiac Fibroblasts and Myofibroblasts

Fibroblasts are the most abundant cells in connective tissue. They produce all components of the extracellular matrix, including protein fibers and amorphous ground substance. They are also essential during wound healing.

Cardiac fibroblasts have been found in all compartments of the heart. They are mainly responsible for the production of major components of the extracellular matrix, including collagen (type I, III, V, and VI), periostin, vimentin, and fibronectin. Thus, fibroblasts create a basic microenvironment for other cell types in the

heart. Moreover, they play a pivotal role in cardiac development, remodeling, and regeneration [4, 5].

It is well-known that cardiac fibroblasts are generated during embryogenesis through a process known as the epithelial-to-mesenchymal transition [6]. The discovery of epicardium-specific genes in the mouse, such as *Tcf21*, *Tbx18,* and *WT1,* strongly support this theory [7].

Despite the fact that fibroblasts have been studied for many years, there is no universal molecular marker used to track them *in vitro* or *in vivo*. This is primarily because all known markers are also expressed by many other cell types within the heart. In pioneering studies, fibroblasts were originally characterized according to their morphological features, proliferation activity, gene expression, and developmental origin [8–11]. This also applied to the fibroblasts of the heart, necessitating the combining of various markers for their identification. Several markers have been used recently to identify cardiac fibroblasts, such as CD90 (also known as Thy1), fibroblast-specific protein 1, discoidin domain receptor 2, fibronectin, vimentin, and collagen types I and III [12]. More recently, it was shown that activated fibroblasts are enriched for fibroblast-activating protein and alpha-smooth muscle actin [13, 14]. Other suitable markers are *Tcf21* and platelet-derived growth factor receptor alpha. These are responsible for fibroblast differentiation and are known to be expressed in adult fibroblasts [15, 16]. However, TCF21 expression is very difficult to detect by immunohistochemistry and platelet-derived growth factor receptor alpha is also expressed by several populations of stem cells within the heart [17]. Another robust marker of cardiac fibroblasts is mEF-SK4, but it must be colocalized with CD31 and CD34 to exclude hematopoietic and endothelial cells [2].

Cardiac fibroblasts play an important role in the synthesis and degradation of cardiac extracellular matrix. They also mediate many physiological and pathological processes that contribute to the structural, biomechanical, biochemical, and electrical properties of the heart.

Cardiac fibroblasts are highly metabolically active cells and they produce an abundance of interstitial collagen, proteoglycans, glycoproteins, growth factors, cytokines, matrikines, and proteases, which influence the composition, function, and remodeling of the extracellular matrix [18]. They form a three-dimensional network for myocytes and other cardiac cells. Moreover, they are involved in the distribution of mechanical forces within the heart [19]. Cardiac fibroblasts respond to changes in the cellular environment caused by physical exercise by modulating integrin expression. This is associated with changes in cell migration [20]. Remodeling and other alterations in the organization of the myocardium are crucial processes that help the heart adapt to changes as a result of physiological or pathological events [21]. Cardiac fibroblasts are the most influential cell types involved in this process. For example, after cardiac injury, fibroblasts are strongly influenced by various bioactive molecules, which promote changes in fibroblast gene expression. They also affect cell migration to damaged regions to promote regeneration and scar formation [22]. Moreover, cardiac fibroblasts differentiate into myofibroblasts, which produce collagen, fibronectin, and contractile proteins [23].

Another major function of cardiac fibroblasts is to express different growth factors, cytokines, and other bioactive molecules. These factors exert autocrine and paracrine effects on the cardiac cells and, thus, promote their proliferation, contraction, and apoptosis [24].

Recent studies demonstrated that cardiac fibroblasts play critical roles in electrical signaling because they possess a high membrane resistance, which makes them excellent conductors. It was shown that fibroblasts are physically coupled to other cells of the myocardium, including myocytes. The cell junctions forming these connections are composed mainly of connexins CX40, CX43, and CX45 [25, 26]. Some studies indicate that cardiac fibroblasts act as bridges connecting myocytes that are normally electrically isolated by connective tissues [27].

Lastly, cardiac fibroblasts have an important role in angiogenesis. They affect this process by releasing several growth factors, such as fibroblast growth factor, vascular endothelial growth factor, and pigment epithelium-derived growth factor [28, 29].

3 Cardiac Telocytes

A telocyte is a type of connecting cell found in various organs in the human body. This includes the heart, which contains telocytes in all cardiac tissue layers. Telocytes create a cellular meshwork throughout the epicardium, endocardium, and myocardium. They can even be found in cardiac stem cell niches [30, 31]. They tend to have a small, rounded appearance, and can sometimes have a spindle-shaped cell body. Most display extremely long cytoplasmic protrusions called telopodes. Each telocyte sprouts 2–5 telopodes, and each of the prolongations can range from dozens to hundreds of micrometers in length with an average thickness of 0.2 μm. Many telopodes form secondary and tertiary branching patterns, and this is what creates the three-dimensional network characteristic of cardiac telocytes. These networks envelop capillaries and connect neighboring telocytes with other tissue types in the heart [32]. Because telopodes are very thin cellular structures, telocytes and their networks must be visualized by transmission electron microscopy (Fig. 8.1) [33, 34] or immunohistochemical staining (Fig. 8.2). Several different antigens are expressed in telocytes, including CD34, CD117 (c-kit), vimentin, and PDGF receptor-alpha and receptor-beta. Unfortunately, all these antigens are also enriched in other non-telocyte cell types. For example, mast cells also express CD117. Therefore, researchers typically use a double-immunolabeling approach to distinguish telocytes from other interstitial cells. Telocytes are often defined as CD34+/vimentin+, CD34+/PDGFR-beta+, or CD34+/CD117+ cells, effectively differentiating them from cardiac fibroblasts [35, 36]. Unfortunately, telocytes cannot currently be distinguished during embryonic development because the maturing progenitors are negative for both CD117 and CD34 [37].

Telocytes are not typically recognized as a distinct cell population. Although the term “telocytes” yields over 240 references in PubMed, there are no official entries

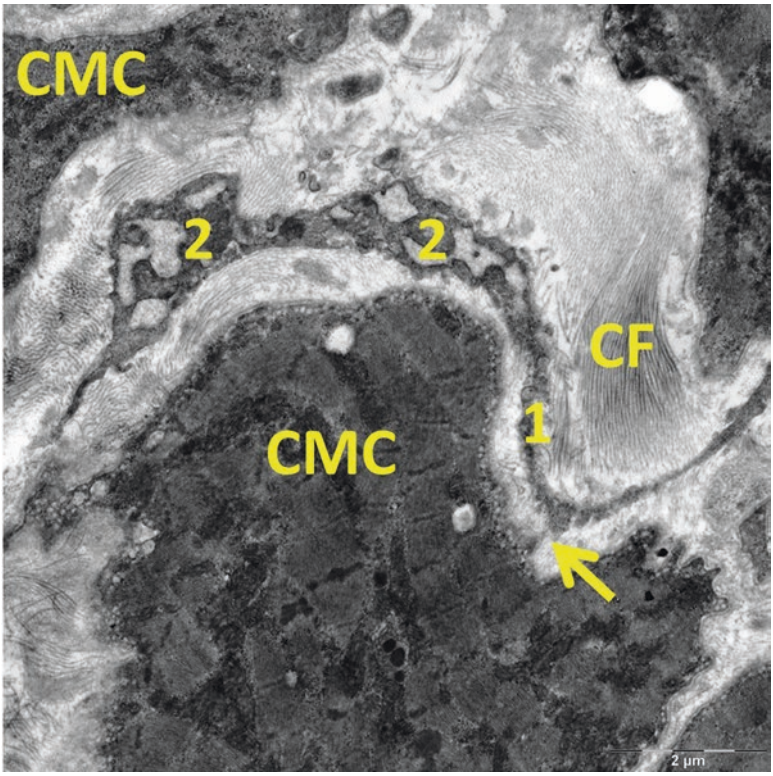

Fig. 8.1 Human myocardium visualized by transmission electron microscopy. Telocytes display long cytoplasmic prolongations in the loose connective tissue with collagen fibers (*CF*) between cardiac muscle cells (*CMC*). Thin segments are called podomers (1), and the thicker dilated segments containing membrane-bound cell organelles are podoms (2). Between the cardiac muscle cell and the prolongation of the telocyte, a direct junction (nanocontact) is visible (*arrow*) (Orig. Magn. 8900×)

for this cell type in *Terminologia Histologica* [38]. Díaz-Flores et al. [39] originally defined the telocyte cell population as stromal fibroblastic cells enriched for CD34. Meanwhile, Ivey and Tallquist [12] used "cardiac telocyte" interchangeably with "cardiac fibroblast." Furthermore, Bei et al. [35] showed that cardiac telocytes have distinct morphologies and immunohistochemical properties when compared to fibroblasts cultured *in vitro*. Rusu et al. [40] also demonstrated that a subpopulation of cardiac telocytes are endothelial derived. Thus, whether telocytes represent a discrete cell population or whether are a special subtype of fibroblasts or endothelial cells remains to be determined.

Cardiac telocytes form a three-dimensional network and are connected to each other at cell-cell junctions. They also form these junctions with other cardiac cell types including cardiomyocytes, cardiomyocyte progenitors, mastocytes, pericytes, immune reactive cells, macrophages, Schwann cells, fibroblasts, and endothelial

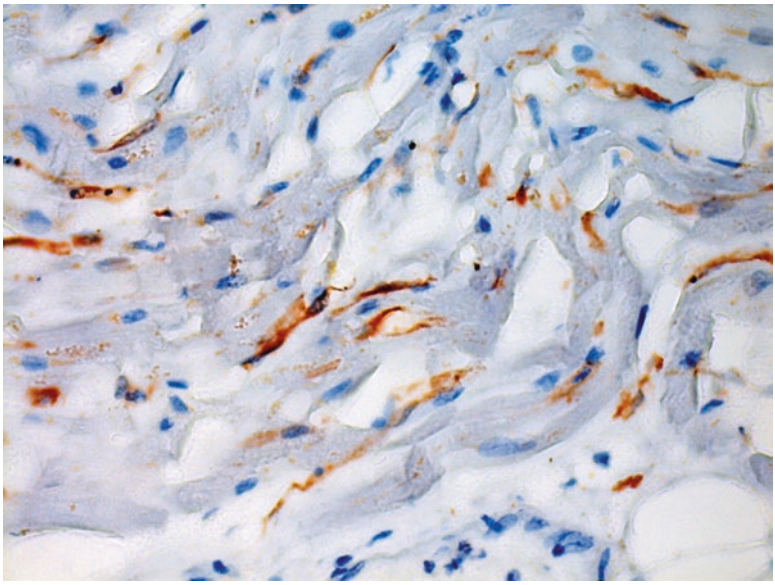

Fig. 8.2 Human myocardium visualized by light microscopy. Spindle-shaped cardiac telocytes (anti-CD117 antibody, *brown* color) between cardiomyocytes and different connective tissue cells inside a scar after a myocardial infarction. Diaminobenzidine was used as a chromogen. Cell nuclei were stained with Mayer's hematoxylin (Orig. Magn. 400×)

cells of the capillaries. Telocytes secrete a range of vesicles containing signaling molecules to facilitate intercellular communication, which has been well-documented *in vitro* [41]. These secreted factors likely regulate nearby cells (especially cardiac stem cells) via paracrine signaling [42].

Telocytes with different morphologies are found in various layers of the heart, and this cell type has discrete functions in different anatomical regions. For example, epicardial telocytes secrete microvesicles as exosomes into the extracellular matrix [43]. Endocardial telocytes are the most abundant cells in the sub-endothelial layer of the endocardium, and they extend telopodes into the myocardium to form direct connections with cells in this region [44]. In fact, the abundance of cardiac telocytes in the subepicardium of rats is significantly higher when compared to the endocardium. The density is higher in the atria compared to the ventricles [45]. Electron microscopy and immunohistochemistry was used to show that telocytes are also present in the valves of the heart [46]. The telocyte meshwork that is formed inside the heart valves likely provides mechanical support and confers flexibility to this tissue, in addition to mediating communication amongst cells.

Faussone-Pellegrini and Bani [37] showed that telocytes are required during prenatal heart development. Immature cardiac muscle cells are interconnected and they are surrounded by telocytes, which provide a three-dimensional scaffold for the myocardium during morphogenesis. After birth, the number of telocytes steadily decrease until adulthood [47].

Telocytes are known to be present in or near stem cell niches in various human organs. This includes the subepicardial cardiac layer [48–50]. In this tissue layer, cardiac stem cell niches harboring cardiomyocyte progenitors are found surrounding the coronary arteries [51]. Each niche contains cardiac progenitors in various stages of differentiation, in addition to loose connective tissue that harbors mast cells, adipocytes, macrophages, fibroblasts, a rich capillary bed, telocytes, and nerve fibers [48]. Recent *in vitro* studies suggest that the telopodes of telocytes form complex scaffolds that are required to organize the myocardium during tissue regeneration [49]. Furthermore, telocytes are often referred to as "nurse cells" for cardiac stem cells because they aid the differentiation and integration of stem cell progenitors into the developing or regenerating heart [50].

A few studies have investigated the contribution of telocytes to the etiology of cardiac diseases. Richter and Kostin [52] demonstrated that there is a reduction in the abundance of cardiac telocytes in patients suffering from end-stage heart failure following transplantation. The loss of telocytes also occurred in rats following myocardial infarction [53]. In subsequent weeks of observation of these rats, cardiac telocytes also failed to migrate into the damaged heart tissue from nearby healthy myocardium, which likely inhibited regeneration of the affected myocardium. Thus, telocytes may contribute to neoangiogenesis following a myocardial infarction [54]. Interestingly, when cardiac telocytes were transplanted into sites of myocardial infarction in rats a reduction in the size of damaged tissue was observed. The animals also experienced significant improvements in heart function. It is therefore possible that the transplantation of healthy cardiac telocytes might increase overall vessel density and decrease myocardial fibrosis in hearts following an infarction [53]. Future cell-based therapeutic approaches of this nature might enhance cardiac regeneration, repair, and protection in patients suffering from various heart diseases [55, 56].

We are aware of only one report linking cardiac telocyte activity to physical exercise. Xiao et al. [57] used a ramp swimming exercise to study the connection between cardiac telocytes function and exercise-induced cardiac growth in mice. It was shown that the number of telocytes increased significantly in the heart following exercise, suggesting that this cell population might modulate the physiology of cardiac stem cells, cardiomyocytes, and/or other endothelial cells. This lends further support to the idea that cardiac telocytes likely enhance cardiac regeneration following an injury or aging processes.

4 Cardiac Adipocytes and Epicardial Adipose Tissue

Adipose cells (fat cells, adipocytes) are specialized cells of the connective tissue that synthesize and store lipids as an energy reserve. Currently, white adipose tissue is considered to not only be an energy-storing organ, but it also acts as:

- an active endocrine organ with an intricate role in systemic homeostasis [58],
- a reservoir for a high number of adipose-tissue derived mesenchymal stem cells [59],
- a regulator of immune responses (e.g., obesity, which is associated with low-grade, sustained, and systemic inflammation, termed obesity-related inflammation) [60].

Epicardial adipose tissue (or epicardial fat) is the adipose tissue depot mainly surrounding the epicardial coronary vessels (Figs. 8.3, 8.4, 8.5, and 8.6). It is a metabolically active part of the heart and it secretes numerous bioactive molecules, such as inflammatory adipokines, growth factors, and cardioprotective factors. A recent meta-analysis demonstrated that epicardial adipose tissue is significantly thicker in patients with coronary artery disease compared to healthy patients [61]. Numerous studies propose that interactions occur between the epicardial adipose tissue and the remodeling myocardium, which underpin the etiology of coronary artery disease, various metabolic syndromes, and atrial fibrillation [62–64]. A number of studies have shown that epicardial adipose tissue causes an overproduction of several pro- and anti-inflammatory cytokines and bioactive substances, including leptin, tumor necrosis factor-alpha, and adiponectin [65, 66]. The protein hormone adiponectin can be produced by other cell types, including cardiomyocytes. Under physiological conditions, its expression level in cardiomyocytes is significantly lower than in adipose tissue. The main source of plasma adiponectin is the adipose tissue [67]. Adiponectin is a protective factor for the heart. It is characterized by its anti-inflammatory, anti-atherogenic, anti-apoptotic, and anti-hypertrophic effects. Many studies show that adiponectin levels decrease in patients suffering from diabetes mellitus, coronary artery disease, hypertension, or dilated cardiomyopathy [68–70]. On the other hand, Takahashi et al. [71] showed that adiponectin is expressed by injured cardiomyocytes in patients with myocardial infarction or dilated cardiomyopathy. Furthermore, Takano et al. [72] suggested that the heart

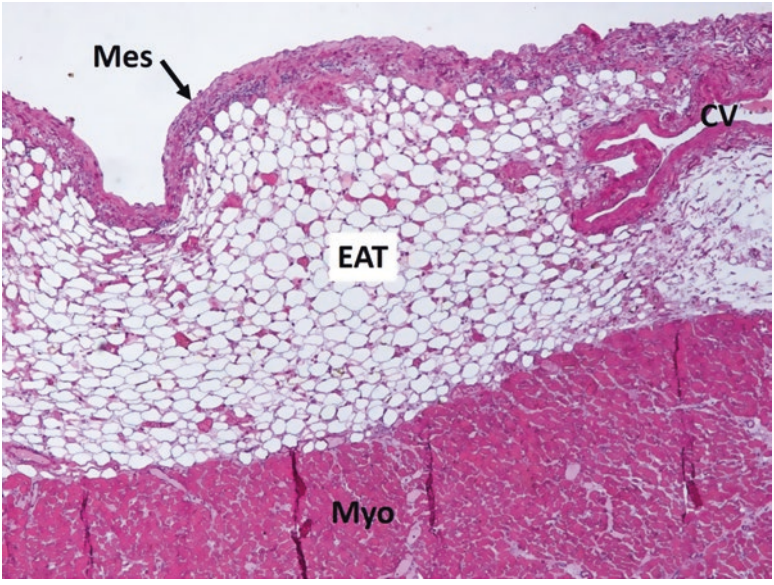

Fig. 8.3 The epicardium of the human heart containing epicardial adipose tissue (EAT) visualized by light microscopy. *Mes* mesothelium, *CV* coronary vein, *Myo* myocardium (H&E stain, Orig. Magn. 50×)

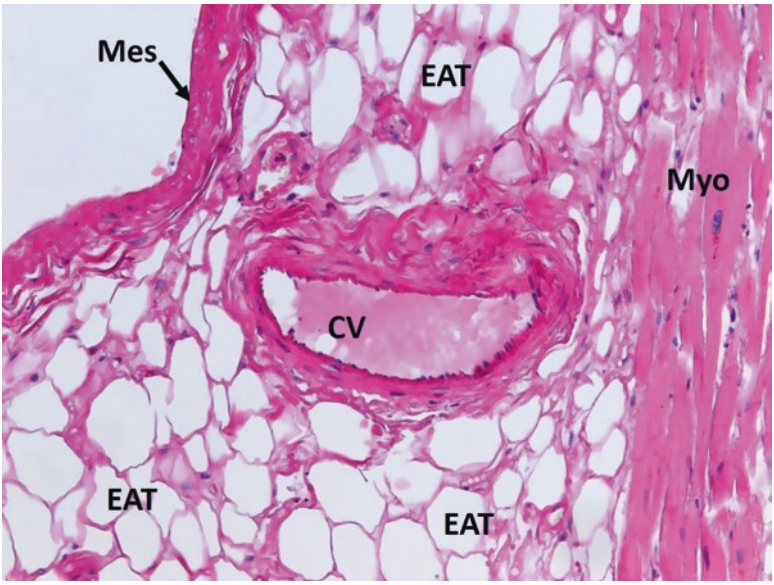

Fig. 8.4 Epicardium of the human heart containing epicardial adipose tissue (EAT) visualized by light microscopy. *Mes* mesothelium, *CV* smaller coronary vein or venule, *Myo* myocardium (H&E stain, Orig. Magn. 200×)

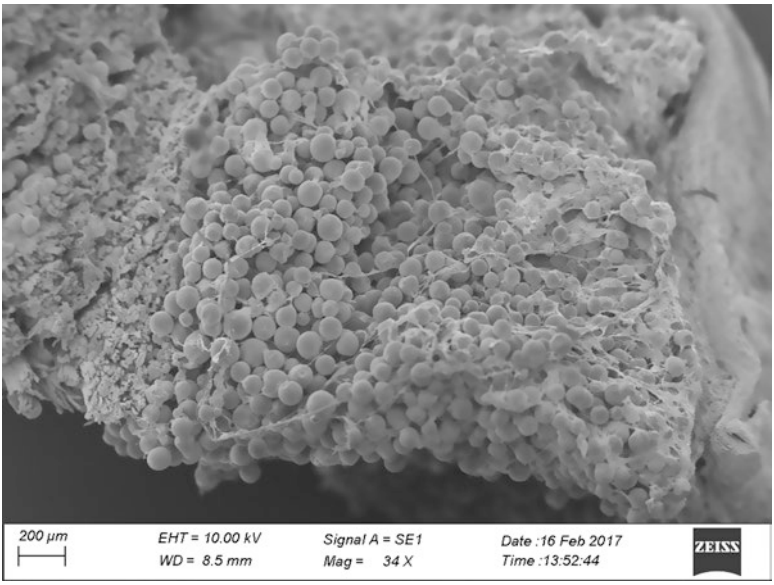

Fig. 8.5 A human heart visualized by scanning electron microscopy showing epicardial adipose tissue (Orig. Magn. 34×)

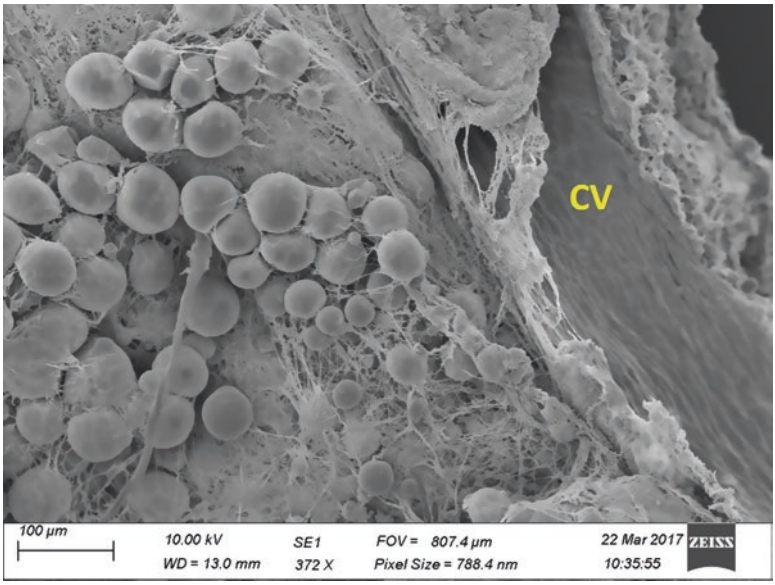

Fig. 8.6 A human heart visualized by scanning electron microscopy. A group of adipocytes from the epicardium surrounded by a delicate network of reticular fibers. The lumen of a small coronary vessel is observed (CV) (Orig. Magn. 372×)

releases adiponectin in response to left ventricular dysfunction, resulting in the elevation of adiponectin plasma levels [73], which is a predictor of mortality in patients with chronic heart failure [74].

It should be noted that the effect of weight loss on the reduction of epicardial adipose tissue is still controversial. For example, Wu et al. [75] examined the volume of epicardial adipose tissue (measured using CT scans) in two groups of overweight or obese patients. The first group underwent bariatric surgery, and the second group participated in a 3-month aerobic exercise and low-calorie diet program. Surprisingly, the epicardial adipose tissue was found to be unaffected by weight loss in both groups of patients. A meta-analysis reported by Rabkin and Campbell [76] suggested that significant epicardial adipose tissue reduction only occurred with improved diet and bariatric surgery, but not with exercise.

5 Cardiac Mast Cells

Mast cells are connective tissue cells of hematopoietic origin. They originate from $CD34^+$ hematopoietic progenitor cells of the bone marrow and differentiate in sites of connective tissue throughout the human body, including the heart. The internationally accepted histological nomenclature, the *Terminologia Histologica* [38], distinguish

two basic types of mast cells. The first type is associated with loose connective tissue of the adventitia of blood vessels (the so-called perivascular mast cells). The second type is localized predominantly in the mucosa (mucosal mast cells), especially in the respiratory and digestive systems [77]. Over the last decades, many scientists have defined another resident population of mast cells: the cardiac mast cells. An increased number of cardiac mast cells have been reported in different chronic or acute diseases of the cardiovascular system, such as dilated cardiomyopathy, hypertension, chronic cardiac volume overload, and myocardial infarction [78]. It is assumed that cardiac mast cells probably participate in myocardial dysfunction and myocardial remodeling, but the precise mechanisms are presently unknown.

Mast cells are easily identifiable in histological tissue sections (Fig. 8.7). They are ovoid with a single spherical nucleus and many cytoplasmic granules. These granules stain intensely and metachromatically (a characteristic change in the color of the applied dye) in the presence of basic dyes, such as toluidine blue or thionine. At the ultrastructural level, the cytoplasmic granules of mast cells are diverse. Some are electron-dense, and others form scrolls or crystals [79]. In recent decades, monoclonal antibodies have been commonly used for immunohistochemical identification of mast cells in tissue sections. Anti-tryptase antibodies represent the "gold standard" for identifying mast cells in human tissues. Tryptase, a trypsin-like serine protease, is specific for mast cells. Other markers enriched in human cardiac mast cells include IgE receptor, CD117 (c-kit), p24 antigen, Pgp-1 homing receptor (CD44), and ICAM-1 antigen (CD54). However, these can also be expressed by

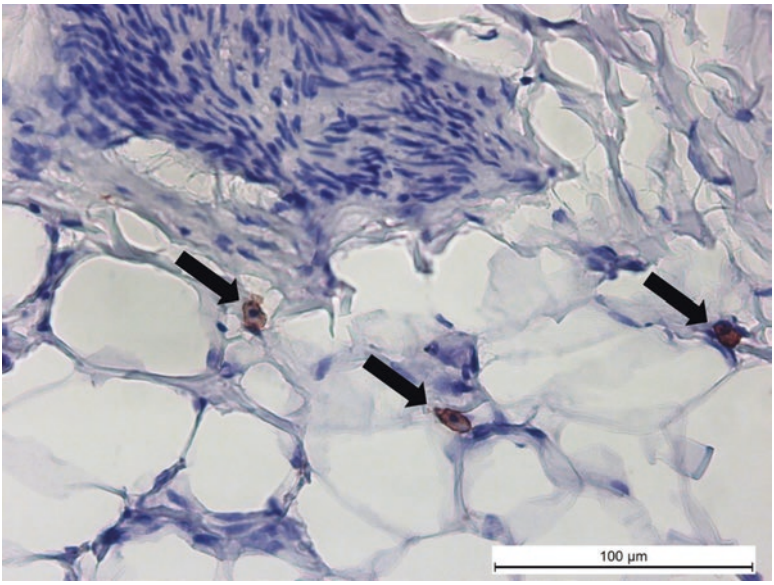

Fig. 8.7 A human heart visualized by light microscopy. *Ovoid-shaped* mast cells (arrows, anti-CD117 antibody, *brown* color) are observed between adipocytes in the epicardium. Diaminobenzidine was used as a chromogen (Orig. Magn. 400×)

many other types of cells and, thus, are not mast cell-specific [80]. For example, CD117 is frequently used to identify not only mast cells, but also cardiac muscle progenitor cells [81] and telocytes [82].

Cardiac mast cells are frequently localized in close proximity to blood vessels. This suggests that circulating antigens or drugs used during disease treatment or diagnostic procedures can easily reach these cardiac mast cells [79]. Mast cells store and release a variety of biologically and pharmacologically active mediators. Some of these, such as histamine, heparin, and serine proteases (tryptase and chymase), are stored in cytoplasmic granules. Other vasoactive and cell signaling mediators, such as leukotriens, interleukins, and prostaglandin D2, are released from the cell membrane during mast cell activation [83]. Furthermore, cardiac mast cells contain and release renin, which initiates local angiotensin formation. This may result in coronary vasoconstriction, arrhythmias, and fibrosis [84].

In general, cardiac mast cells are responsible for immune reactions and inflammation. Mast cells manufacture a wide variety of proteases, cytokines, growth factors, and vasoactive substances that may influence myocardial remodeling [85]. Mast cell proteases are capable of activating collagenase, and other mediators, such as tryptase and chymase, have the ability to activate metalloproteinases [86]. In recent years, numerous interesting studies have been published that focused on the role of cardiac mast cells during the pathogenesis of cardiac diseases (mostly in animal models). For example, Levick et al. [87] demonstrated a causal relationship between cardiac mast cells and the development of left ventricular fibrosis in response to hypertension. A significant increase in cardiac mast cell density was also observed to correlate with cardiac hypertrophy and heart failure. For these reasons, mast cells may contribute to the development of cardiac hypertrophy and heart failure [88]. Finally, mast cells may play a role in the etiology of eosinophilic coronary periarteritis, a rare eosinophil-induced inflammation associated with spontaneous coronary artery dissection and sudden cardiac death [89].

The activity of mast cells can be modulated be exercise. In a rat model, for example, Phungphong et al. [90] found that regular exercise had a protective effect on the heart by inhibiting the degranulation of mast cells.

6 Cardiac Mononuclear Phagocytic Cells

In general, cardiac immune cells are gaining interest for the roles they play in pathological remodeling in a number of cardiac diseases [91]. These immune cells include T-lymphocytes and macrophages. B-lymphocytes are less numerous in the human myocardium [92].

Macrophages belong to the mononuclear phagocytic system and are part of the innate immune system. They play a role in the maintenance of normal tissues by ingesting dead cells and cellular debris and breaking them down with lysosomal enzymes. Macrophages participate in the immunological response. They are the first line of defense against infection. There are two predominant hypotheses regarding

the origin of macrophages. The traditional model states that blood monocytes give rise to all tissue macrophages. The second hypothesis assumes a prenatal colonization of tissues by resident macrophages derived from embryonic yolk sac progenitors. These macrophages persist throughout adulthood and self-renew without input from circulating monocytes [93–95].

Macrophages secrete a variety of cytokines, pro-inflammatory molecules, and trophic mediators. Some of these have been suggested to inhibit apoptosis in hypoxic cardiomyocytes or promote neonatal heart regeneration [96]. In various cardiac diseases, such as ischemic heart disease and idiopathic dilated cardiomyopathy, the expansion of macrophage populations occurs [97]. Cardiac macrophages may contribute to tissue remodeling during chronic pressure-overload heart failure or heart fibrosis through the activation of myofibroblasts [98, 99]. The role of macrophages in inflammation after a myocardial infarct indicates that these cells are absolutely necessary for adequate wound healing and scar formation [100].

There is only one scientific article that describes the link between cardiac macrophages and physical exercise. In a study by Botta et al. [101], the infiltration of the hearts of diabetic mice by F4/80+ macrophages was attenuated by exercise, which consisted of animals running on a motorized exercise wheel system.

7 Cardiac Endothelial Cells and Pericytes

Endothelial cells resemble simple squamous epithelial cells. They have their own basal lamina and line blood vessels. The internationally accepted *Terminologia Histologica* describes them as an epithelial tissue [38]. On the other hand, they are of mesodermal origin and can produce collagen type IV. Therefore, endothelial cells are also considered to be connective tissue cells. Inside the heart, there are two different populations of cardiac endothelial cells:

- vascular endothelium (lining the luminal surface of the coronary vessels; Fig. 8.8)
- endocardial endothelium (a continuous monolayer of cells that line the cavities of the heart; Fig. 8.9).

The differences between these types of endothelial cells are apparent only on the ultrastructural level. For example, endocardial endothelial cells have Weibel-Palade bodies in their cytoplasm, which contain von Willebrand factor [102]. Additionally, endocardial endothelium has a different cell shape, cytoskeletal organization, and permeability than vascular endothelium [103]. From an embryological point of view, vascular endothelium originates from the epicardium, and endocardial endothelium originates from the cardiogenic plate [104].

Both vascular and endocardial endothelial cells play a role in controlling the contractility of cardiomyocytes by releasing various biologically active autocrine and paracrine agents. Cardiac endothelial cells produce nitric oxide, endothelin-1, prostaglandin I (2), angiotensin II, and other factors [104, 105]. All of these sub-

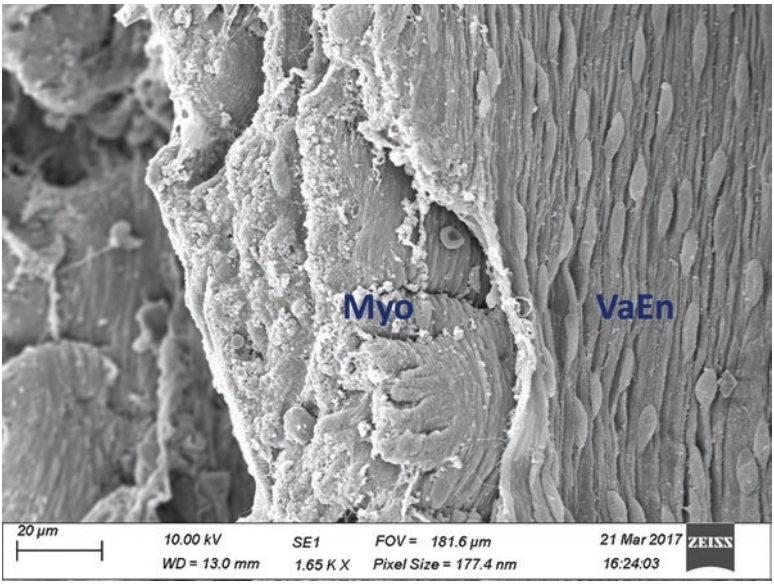

Fig. 8.8 A human heart visualized by scanning electron microscopy. The lumen of a small thin-walled vessel (probably a venule) lined with vascular endothelium (VaEn) is observed. The endothelial cell nuclei protrude into the lumen. Some cardiac muscle cells of the myocardium (Myo) are also observed (Orig. Magn. 1650×)

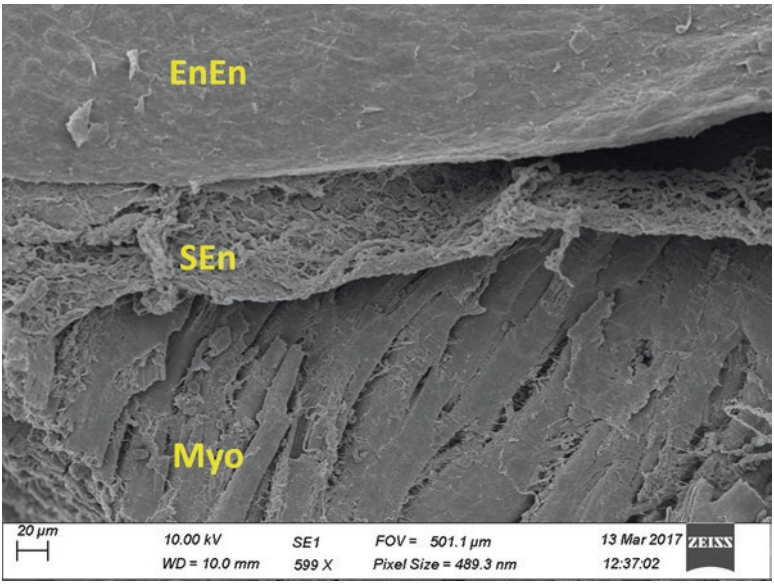

Fig. 8.9 A human heart visualized by scanning electron microscopy. Surface endocardial endothelium is observed beneath a subendothelial layer of loose connective tissue and cardiac muscle cells in the myocardium (Myo). (Orig. Magn. 599×)

stances directly influence cardiac metabolism, growth, contractile performance, and rhythmicity of the adult heart.

In recent years, endothelial progenitor cells that contribute to angiogenesis have been identified as a circulating cell population in the peripheral blood. They are derived from the bone marrow [106]. Endothelial progenitor cells are rare in the circulation, but they can be mobilized into the circulation from the bone marrow by vascular trauma or some types of cytokines. They may be associated with some degenerative diseases, such as progressive progenitor cell deficits that may contribute to the development of atherosclerosis [107]. The study of Rehman et al. [108] demonstrated that exercise can acutely increase two distinct cell populations that are known to be involved in angiogenesis and endothelial repair. These include circulating endothelial progenitor cells, which may supply new endothelial cells to the vasculature, and circulating angiogenic cells, which secrete growth factors that promote endothelial growth and angiogenesis. Furthermore, Adams et al. [109] confirmed that there is an increase in the number of circulating endothelial progenitor cells in patients who undergo exercise-induced myocardial ischemia after exercising on an electronically braked bicycle. It seems that an ischemic stimulus may trigger the release of endothelial progenitor cells from the bone marrow in the peripheral blood. These results are in accordance with a recently published meta-analysis of 16 different studies [110]. This meta-analysis found that exercise training improved endothelial function in patients suffering from heart failure. It is likely that bot, acute and chronic exercise have the potential to mobilize endothelial progenitor cells, which are important players in endothelial repair.

Pericytes (Rouget cells) are cells closely encircling endothelial cells in capillaries and microvessels. They have a branched, flattened cytoplasm and oval nuclei. The antigenic profile is important for immunohistochemical identification, and it includes the expression of CD146, PDGFR-beta, and alkaline phosphatase [111]. In general, pericytes are involved in the preservation of vascular homeostasis, including the regulation of blood flow, angiogenesis, structural stabilization of the vasculature, and vascular permeability [112]. However, the functions of pericytes are varied. The initial hypothesis that pericytes are only supportive perivascular cells can now be considered obsolete. These cells should be considered to be heterogeneous, tissue-specific, and multipotent populations with myogenic, osteogenic, chondrogenic, and adipogenic potentials. In myocardial ischemia, pericytes have been shown to be involved in fibrosis and scar formation [112]. In skeletal muscle, pericytes accumulate in muscle as a type of mesenchymal stem cells and they contribute to the formation of new muscle fibers and vessel remodeling following exercise (which increases the diameter of vessels and arteriolar density) [113].

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Part IV
Exercise Benefits the Heart: Basic Science Evidence

Chapter 9
Myocardial Infarction and Exercise Training: Evidence from Basic Science

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Abstract In 2016, cardiovascular disease remains the first cause of mortality worldwide [1]. Coronary artery disease, which is the most important precursor of myocardial infarction (MI), is the main component of total cardiovascular mortality, being responsible for approximately seven million of deaths [1]. In approximately 20% of infarcted patients, MI is recurrent in the first year after the event [2]. Moreover, among cardiovascular disease, coronary artery disease accounts for the most increased index of life years lost due to morbidity and/or mortality [1]. Sedentarism highly contributes to cardiovascular disease burden, especially for coronary artery disease, and is also one of the MI risk factors [3]. For many years, it was recommended to avoid physical activity after a cardiovascular event; nowadays, it is a consensus that exercise training (ET) should be part of cardiac rehabilitation programs. There is increasing evidence confirming that, when adequately prescribed and supervised, ET after MI can prevent future complications and increase the quality of life and longevity of infarcted patients [4, 5]. ET after MI follows international specialized guidelines; however, there are different protocols adopted by several societies worldwide in cardiac rehabilitation [6], and there is still lack of information on which type and regimen of exercise may be the ideal after MI, as well as how these exercises act to promote beneficial effects to cardiovascular and other organic systems. Thus, experimental studies are important contributors to elicit mechanisms behind clinical results, and to test and compare different ET protocols. Therefore, exercise prescription can be optimized, individualized, and safely practiced by patients. In this chapter, we present a brief review of MI pathophysiology followed by an updated discussion of the most relevant discoveries regarding ET and MI in basic science.

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1 Pathophysiology of Myocardial Infarction

Coronary artery disease is characterized by the formation of an atherosclerotic plaque following a long-term and complex process [7]. Briefly, when the atherosclerotic plaque suffers a rupture, the disruption of the endothelium stimulates a coagulation process, which results in the formation of a thrombus. MI occurs when the thrombus occludes coronary blood flow and the surrounding myocardium area lacks oxygen supply, thus leading to the necrosis of the cardiac tissue. Depending on the level of the occlusion, the extent of the necrotic area, and the presence of collateral circulation, MI can be fatal or not.

When the heart survives to the ischemia, several events occur at molecular, cellular, neurohumoral, hemodynamic, and morphological levels. Adaptive stimuli start at early (until 72 h after MI) and late stages (more than 72 h), going through a pathological remodelling process. As MI occurs, inflammation takes place in the necrotic area, where matrix metalloproteinases (MMPs) stimulate the disintegration of intermyocyte collagen, resulting in the loss of support tissue. The cardiac wall of the infarcted area gets thinner and the ventricular cavity dilates, a phenomenon known as infarct expansion [8, 9]. Functionally, because of myocyte loss, there is a decrease in ejection volume, thus increasing preload due to elevated diastolic final volume, and an increase in ventricular wall stress. The elevated cardiac wall stress is a stimulus to serial myocyte replication, resulting in ventricular hypertrophy [9, 10]. The survival heart can continue its pumping function facing this new morphofunctional pattern for a long term; nevertheless when cardiac hypertrophy cannot compensate the increased ventricular volume, it suffers progressive ventricular enlargement and dysfunction [10].

There are several mechanisms which are altered after MI as cause or consequence of the pathological remodelling process. These altered mechanisms can be seen in both, humans and experimental models, and encompasses hemodynamics, the autonomic nervous system, the baroreflex sensitivity, the renin-angiotensin-aldosterone system (RAAS), the sarcoplasmic reticulum calcium transient, the beta-adrenergic pathway, and oxidative stress [9, 11, 12]. Among others, these mechanisms are the main targets of pharmacological and non-pharmacological therapeutic strategies to improve cardiac prognosis after MI.

2 Experimental Models of Myocardial Infarction

Studies using rats and mice as animal models are conducted to better understand the mechanisms involved in the pathophysiology of MI, to study cardioprotective interventions, as well as to evaluate the processes that occur during and after myocardial

remodelling, allowing the development of possible therapeutic approaches [13–15]. In the following sections, the reader will find a brief description of the most used animal models of MI in the context of ET adaptations.

2.1 Left Anterior Descending Coronary Artery Ligation (LAD)

LAD is one of the most used models of MI, since its repercussion on the organic systems is similar to the observed in human beings. In fact, LAD-induced MI causes autonomic dysfunction, impairment of baroreflex sensitivity (BrS), impairment on cardiac functioning and morphology, exercise intolerance, increase in proinflammatory cytokines (PICs), among others [13, 14, 16–19].

LAD is performed after animal anaesthesia (ketamine (80 mg/kg) and xylazine (12 mg/kg) are commonly used as anaesthetics. After intubation, the animals are positive-pressure ventilated with room air at 2.5 mL, 65 strokes/minute with a pressure-cycled rodent ventilator. For induction of MI, a 2-cm left lateral thoracotomy is performed in the third intercostal space, and the left anterior descending coronary artery is occluded with a nylon (6.0) suture at approximately 1 mm from its origin below the tip of the left atrium. When experimental protocols using this models are performed, the studies usually subject a control group to sham surgery, where animals receive the same invasive interventions but are not subjected to myocardial ischemia [13, 14, 16–19].

2.2 Ischemia-Reperfusion Model

Ischemia-reperfusion model has been used, generally, to study the effects of reperfusion stress on reactive oxygen species (ROS) behaviour [20, 21].

After anaesthesia and intubation for mechanical ventilation, the rats are subjected to a procedure similar to that described previously in LAD model, in which the proximal left anterior descending artery is identified and the artery is then transiently ligated (or can be tied by a slipknot) using a 6.0 nylon suture for a 30-minute ischemic period, but without exteriorization of the heart. To allow cardiac reperfusion, microsurgical scissors are used to cut the knot in the ligature (or by releasing the slipknot), made by the 6.0 nylon suture, 30 minutes following ligation of the artery. In sham control rats, the procedure is identical, except for the artery which is not transiently ligated. The outflow is then briefly (1–2 s) pinched off on the respirator to allow re-inflation of the lungs. The chest retractor is then removed and the ribs are drawn together using a 2.0 nylon suture with an interrupted suture pattern. Once the ribs are closed, the outflow of the ventilator is again briefly (1–2 s) pinched off to ensure proper breathing. The skin is closed using 6.0 nylon sutures with a continuous suture pattern [22].

2.3 Myocardial Infarction Induced by Isoproterenol

The induction of MI is performed through subcutaneous administration of isoproterenol at a dose of 150 mg/kg/day diluted in 2 ml of saline on two consecutive days with an interval of 24 h between applications. The false induction of MI in the sham group is performed by subcutaneous administration of 2 ml of saline on two consecutive days, also with an interval of 24 hours between applications [23].

3 Physical Activity and Exercise Training in the Context of MI: General Concepts

Before beginning data presentation about the protective or regenerative effects of physical activity (PA) and/or ET on MI, it is necessary to clarify the main concepts and recommendations involving both kinds of body movement. In fact, although similar, PA and ET have different concepts and, generally, cause distinct body adaptations. Moreover, these tools are applied in different contexts, depending on the purpose chosen by the healthcare team (e.g., physical educator, physiotherapists, nurse, physician) after evaluation and patient agreement.

The American College of Sports Medicine (ACSM) has defined PA as any body movement performed in response to voluntary muscle contraction that increases energy expenditure [24]. Thus, it is important to understand that blinks or shivering are not considered PA, even if they are types of body movement. On the other hand, walk for some minutes in the park talking with a friend is a PA, once the contraction of leg muscles is voluntary and the energy expenditure increases exponentially from baseline levels. In turn, ET refers to a more elaborated concept, which concerns a planned and structured body movement aimed to improve one or more physical capacities. ET has different designs, and can be introduced as, for example, aerobic and strength/resistance exercise, swimming training, yoga, among others, depending on the approach.

The American Heart Association (AHA) describes PA as an important tool to be used in the prevention of a variety of pathologies, as hypertension, diabetes mellitus type II, obesity, as physical inactivity is strongly associated with cardiovascular disease risk factors, morbidity and mortality [25, 26]. Moreover, AHA strongly encourages the inclusion of PA in the lifestyle changes of patients who aim to decrease cardiovascular disease risk factors [25, 26]. General recommendations indicate that adults should achieve, at least, 150 minutes of moderate-intensity activity or 75 min of vigorous-intensity activity per week to prevent cardiovascular disease [25, 26].

In the context of cardiac rehabilitation and secondary prevention, ET is generally the used approach. Surprisingly, epidemiological data about its preventive effects are not elucidated, since prescription of ET are dependent on some factors, as exercise volume, intensity, cadence, which are difficult to control in observational studies (i.e., follow-up). However, the effects of ET on cardiovascular disease risk

factors are widely elucidated in clinical trials, experimental studies and observational studies (i.e., cross-sectional). For now, ET should compose the rehabilitation programs of cardiac patients, since its practice has been demonstrated to improve exercise tolerance, quality of life, functional capacities and job-related physical tasks, as well as decrease cardiovascular risk factors and cardiac mortality [26].

4 Physical Activity and Myocardial Infarction

In animal studies, PA can be mimicked by the voluntary run performed by the animals in a running wheel during a determined period. In the context of MI, authors have studied the posterior and previous plus posterior effects of PA on cardiac remodelling and functioning in infarcted mice. However, just few evidence have been published in this issue and more experiments are necessary.

In this sense, Bito et al. [27] studied the effects of PA posterior to MI on cardiac remodelling of infarcted mice. Thus, after MI, animals had free access to the running wheel during 8 weeks. To investigate cardiac remodelling, myocytes from the non-infarcted left ventricle were isolated and investigated regarding morphological and functional aspects. After several analyses, authors observed that sedentary mice showed a cardiac remodelling phenotype, characterized by increased heart weight-body weight ratio and cell width. PA was not effective to inhibit such morphological alterations and both groups presented similar results. In turn, cell shortening elicited by electrical stimulation, which was decreased in infarcted sedentary mice, was restored in the cardiomyocyte of infarcted mice which had access to the running wheel [27]. Further analyses showed that calcium transient was increased in animals from the PA group due to an elevated capacity of Ca^{2+} removal by Na^{+}-Ca^{2+} exchanger (NCX) [27].

In turn, Puhl et al. [28] not only studied the posterior effects of PA on MI, but also the previous effects. Firstly, mice could voluntary run in a running wheel for 6 weeks mimicking a PA context. After this period, mice underwent experimental MI and, 5 days after the surgery, were allowed to use the running wheel for more 4 weeks. Similar to Bito et al. [27], results indicated that PA did not modulate MI-induced cardiac hypertrophy, since increased organ weight and cardiomyocyte diameter were equally observed in both PA and sedentary groups. However, histological and magnetic resonance imaging analyses indicated that PA decreased collagen content and scar formation of the whole left ventricle and in the scar region after MI, as well as partially inhibited the formation of apical aneurysms associated with left ventricle dilation. Authors also observed decreased MMP activity and mRNA expression of proinflammatory citokynes (i.e., TNF-α, IL-6 and IL-1β). These alterations on inflammatory state seem to have impacted cardiac morphology, as the mRNA expression of TNF-α was positively correlated with infarct size and collagen mRNA expression in sedentary mice, whereas this phenomenon was blunted and not showed in the exercised group.

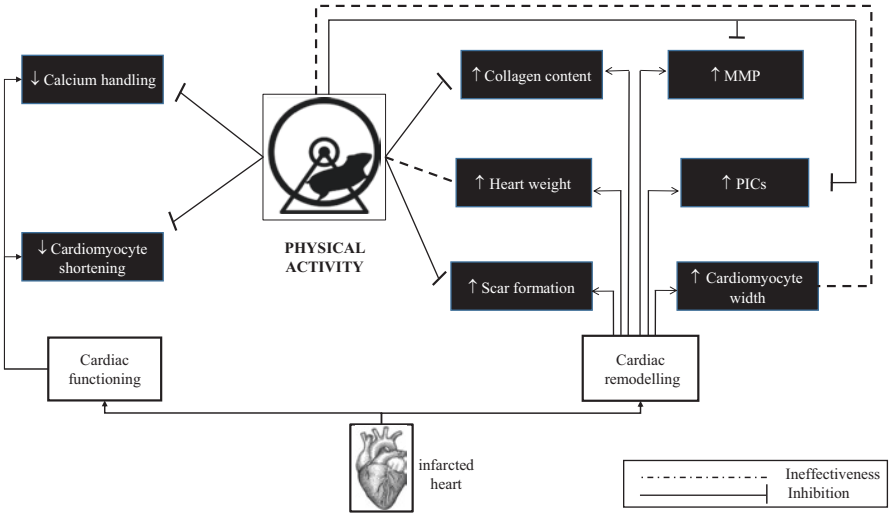

Fig. 9.1 Main effects of physical activity (PA) in experimental myocardial infarction (MI). In rodents, PA can be studied using a voluntary running wheel where animals can exercise. *MMP* matrix metalloproteinases, *PICs* proinflammatory citokynes

It is important to mention that mice from Bito et al. [27] ran in a larger average daily distance (~11 km/day) than animals from Puhl et al. (~5 km/day) [28]. However, animals from Puhl et al. [28] ran during 10 weeks versus 8 weeks from Bito et al. [27]. Therefore, more evidence about the effects of PA on experimental MI are still necessary. Figure 9.1 depicts the main alterations induced by PA in experimental models of MI.

5 Exercise Training and Myocardial Infarction

As aforementioned, ET has been used as a powerful tool in cardiac rehabilitation programs, collaborating to improve prognosis, once its practice can revert some impairments observed after MI. Either aerobic (endurance) and resistance ET can be prescribed as part of the rehabilitation program. Nevertheless, the majority of the experimental studies were conducted having the aerobic training (performed on a treadmill) as choice. This may be due to a higher similarity to cardiac rehabilitation programs for humans, as aerobic training is predominant. Other methods of ET have also been studied in experimental models of MI as detailed in this section.

The benefits of ET on cardiac remodelling seem to occur in both phases: early and late. The early remodelling phase is characterized by the expansion of the infarcted area induced by degradation of the structural collagen presented in the extracellular matrix (ECM) by MMPs, which are secreted by the immune cells in response to MI-induced inflammation [8, 29]. In addition, in an attempt to preserve cardiac out-

put, biological system alters the autonomic control of the heart and vessels favouring the sympathetic activity, thus increasing cardiac chronotropism (frequency) and inotropism (strength), as well as the activity of molecules associated with adrenergic control as catecholamines and RAAS [8]. Even if such alterations act beneficially in the first hours after MI – collaborating to maintain blood perfusion to the tissues, and, consequently, nutrients delivery - over time these compensatory alterations will cause several malefic alterations in cardiac functioning and structure [8, 30].

ROS activity is increased from the early remodelling phase to the late remodelling phase in response to cardiac ischemia with or without reperfusion [21, 31, 32]. ROS synthesis and release are induced by several elements showed in the ischemic myocardium including, but not limited to proinflammatory citokynes and RAAS [29, 30, 32]. Once activated, ROS pathway will act triggering alterations on cardiomyocyte, collaborating, for example, with cardiac hypertrophy induced by proinflammatory citokynes and RAAS [30]. On the other hand, activity of different elements of the antioxidant milieu (e.g., vitamin E, catalase, superoxide dismutase [SOD]) seems to successfully inhibit such alterations induced by ROS [21, 30]. Indeed, the myocardium of transgenic animals overexpressing SOD shows abolished free radical generation, ameliorated recovery of contraction function and lower infarcted area after ischemia-reperfusion, in comparison with wild-type animals [20, 21].

Even if there are no evidence indicating the effects of ET posterior to MI in the early remodelling phase, an isolated result showed that 12 weeks of low-to-moderate aerobic exercise performed during 50 min, 5 days per week previously to MI induced by isoproterenol was effective to decreased infarction degree and ROS expression and activity in the heart of the trained rats in comparison with sedentary animals. Concomitantly, the authors observed decreased activity of antioxidant enzymes, as superoxide dismutase and catalase in the heart of sedentary rats. However, ET could attenuate this phenomenon [31]. However, the study did not accomplish additional analyses, as cardiac functioning, and inferences about the data are limited.

As abovementioned, besides its association with ROS, proinflammatory citokynes have a marked influence on MMPs [29, 33]. Immediately after myocardial injury caused by ischemia, neutrophils migrate to the MI area and recruit MMPs, which induce collagen breakdown [29]. This phenomenon will induce fibroblast migration and, posterior, differentiation in myofibroblasts, which are crucial to fibrotic tissue formation (i.e., scar) [29, 33]. In summary, myofibroblasts causes accumulation of matrix collagen fibers, glycoprotein fibronectin, type III collagen replaced by type I collagen, formation of the fibrin clot and collagen-based scar [33].

Experiments have been indicating that ET can modulate this response, since ET performed previous and after MI reduces the components of the ECM remodelling. Bozi et al. [34], for instance, observed that 8 weeks of moderate ET previous to experimental MI decreased collagen content in the heart of rats. In relation to post-MI effects, Xu et al. [22] and Yengo et al. [35], showed that moderate ET performed during 8 and 10 weeks, respectively, decrease the collagen volume and content in the infarcted area. Moreover, further analyses of Xu et al. [22], evaluated non-reducible collagen cross-linking (hydroxylysyl pyridinoline, HP) content - a marker

of the strength and maturity of the collagen – and observed normalized levels in the non-infarcted area and in the right ventricle of MI rats after ET [22]. None of the studies investigated the possible mechanisms associated with ameliorated collagen deposition in the heart of MI rats.

In front of this lack, inferences can be made by other studies which used similar ET programs. Rodrigues et al. [14], showed decreased TNF-α protein content and TNF-α/IL-10 ratio in the left ventricle of MI rats subjected to 3 months of moderate aerobic ET [14]. In addition, a study from Melo et al. [36], seems to offer a better elucidation trough microRNA analyses, once the authors report that swimming ET increases MiRNA-29a, b and c expression on border region and remote myocardial of MI rats. These results were associated with decreases on collagen expression and content in ~45% [36].

Once ET showed effectiveness to decrease the percentage of collagen deposition, as well as normalized the levels of HP in the heart of MI rats, several studies hypothesized that ET could revert and normalize scar formation after MI. Most studies have been evaluating scar formation through ventricular wall thickness measurement by echocardiographic analyses. This evaluation has been demonstrating strong correlation with histological data. Results regarding scar formation are uncertain and can be exercise-dependent, since evidence indicate that after treadmill ET it is possible to observe an increase in wall thickness [13, 14, 17], whereas 10 weeks of swimming ET (60 min; 5 days/week) seems not to proportionate the same effect [36].

A last repercussion elicited by collagen degradation is wall thinning ventricular dilation, which strongly elevates systolic and diastolic wall stress [8]. In conjunction with other cellular signalling mechanisms associated with cytokines, RAAS, increased sympathetic nerve activity, catecholamines, and fetal genes, this phenomenon induces pathological cardiac hypertrophy.

ET has been extensively studied in this context, believed as a stress capable to counteract the pathological cardiac signalling triggered by MI. Regarding wall thinning ventricular dilation, studies have demonstrated that ET can increase ventricular dilation [13, 14, 16, 17, 37, 38]. However, different from post-MI pathological remodelling, the ejection fraction is also increased indicating a physiological remodelling [13, 14, 16, 17, 37, 38].

When studying the effects of ET on MI-induced cardiac hypertrophy, data have been demonstrating that ET can attenuate cardiac remodelling. Such as in the context of wall ventricular dilation, cardiac hypertrophy seems to be observed in conjunction with data from cardiac functional analyses. In the experiment of Bozi et al. [34], for example, rats underwent 8 weeks of moderate ET (5 days/week;) previous to experimental MI. Animals were kept alive for 15 days after MI surgery and, then, euthanized. Results demonstrated that MI rats showed left ventricular remodelling, indicated by increased heart weight (HW) and HW-body weight (BW) ratio. Interestingly, ET rats also showed elevated HW and HW-BW ratio concomitantly with greater myocyte length and width than sedentary-MI [34]. However, contrary to sedentary MI rats, trained MI rats showed elevated cardiac functioning [34].

Autonomic dysfunction is also an important issue observed after MI, which has been associated with cardiac inflammation, remodelling and functioning, as well as strongly associated with several poor outcomes, including more increased mortality

[11, 18]. Indeed, in a classical study of La Rovere et al. [39], impaired baroreflex sensitivity (BrS) increased the risk of mortality in MI patients. On the other hand, ET attenuated cardiac mortality in a 10-year follow-up [4].

Several evidence from animal studies have been demonstrating that ET improves BrS and normalizes autonomic dysfunction – favouring parasympathetic activity – in MI animals [16]. In addition, after MI, it is possible to observe cardiac sympathetic nerve sprouting associated with imbalance of adrenergic receptor (AR) [40]. The sympathetic activity maintained over time causes downregulation of the β1-AR, desensitization of β2-AR, and upregulation of β3-AR [40]. In the experiment of Chen et al. [40], ET was capable to normalize autonomic control and AR balance. Furthermore, in conjunction with ameliorated autonomic functioning, authors observed improvement of ventricular function, regional blood flow, decrease on proinflammatory cytokines, and reduced mortality [13, 14, 16].

ET has been shown effective to improve cardiac hemodynamics and functioning after the ischemic event [13]. It is important to highlight that studies in animal models have shown that the earlier the exercise program begins, the greater were the benefits in ventricular remodelling due ameliorated cardiomyocyte proliferation, angiogenesis and reduced apoptosis in cardiomyocytes [40]. However, the better "window" to start ET in rodents seems to be after 1 week of MI induction [13, 14, 16] since rats subjected to ET in the first days after MI showed elevated mortality [28].

Regarding the protective effects of ET, studies have shown that ET previous to MI can protect cardiac function after the event. In fact, rats submitted to a swimming protocol and subsequently induced to MI showed preserved left ventricle function, indicated by left ventricular end-systolic diameter, left ventricular end-diastolic diameter, and left ventricular shortening fraction [37, 38]. Moreover, results from treadmill ET added data and indicated that previous MI exercise can also improve echocardiographic parameters, prevent BrS impairment, as well as autonomic dysfunction [17]. Results from experiments performed after MI are not different and showed ameliorated cardiac functioning in MI rats after ET (50–70% VO_{2max}, for 1 h a day, 5 days per week) [13, 14, 16, 17].

Lastly, results have demonstrated to be controversial in relation to the effects of ET on MI area. In summary, MI area is an evaluation that quantifies cardiomyocyte akinetic and kinetic area. Several studies have been demonstrating the effectiveness of ET to cause significant decrease on MI area, evaluated by echocardiographic (i.e., MI akinetic area) and histologic (i.e., fibrosis score) analyses [13, 14, 16, 19]. However, this phenomenon is not observed after swimming ET [36].

During the period of cardiac rehabilitation, some events can make the patient stop the practice of ET avoiding the ideal adherence to the rehabilitation program, such as vacation, travels, economical issues, or even the desire to "take a break", although it is not advisable. In this sense, Barboza et al. [13] and Rodrigues et al. [17] investigated whether 1 month of detraining could reverse the beneficial effects of moderate ET after MI. Results showed that the reduction in MI area, fibrosis score, cardiac morphology and functioning, BrS, proinflammatory state and survival observed after ET did not vanish after 1 month of detraining in rats [17]. However, longer breaks were not studied.

Resistance ET, also called as strength training, is being increasingly prescribed to patients with cardiovascular complications, from hypertensive to heart failure patients. Indeed, several evidence have been demonstrating that resistance ET can participate in conjunction with aerobic ET, or even alone, in the control of blood pressure values in hypertensive patients [41, 42]. Regarding MI, data from basic research are still very limited.

In front of this lack in the literature, Grans et al. [19] subjected MI-rats to 12 weeks of low to moderate (40–60% of the maximum strength) resistance ET program. After the resistance ET program, differences were not observed in the MI size between the infarcted groups. Further analyses showed similar increase in left ventricle mass and relative wall thickness – an indicator of scar formation – in trained groups (i.e., non-MI and MI rats), with significant differences observed between trained-infarcted and sedentary-infarcted rats, in favour of the trained group. These data suggest a positive cardiac remodelling elicited by resistance ET in MI rats. To verify whether data of cardiac function could corroborate with morphological data and indicate a beneficial physiological hypertrophy after resistance ET, the cardiac function was analysed. However, results did not demonstrate alterations on ejection fraction [19]. Moreover, data demonstrate ameliorated autonomic function, indicated by a decrease in both cardiac and vascular sympathetic modulation, concomitantly with an increase in the parasympathetic modulation [19]. Therefore, more evidences regarding the effects of resistance ET on MI-induced

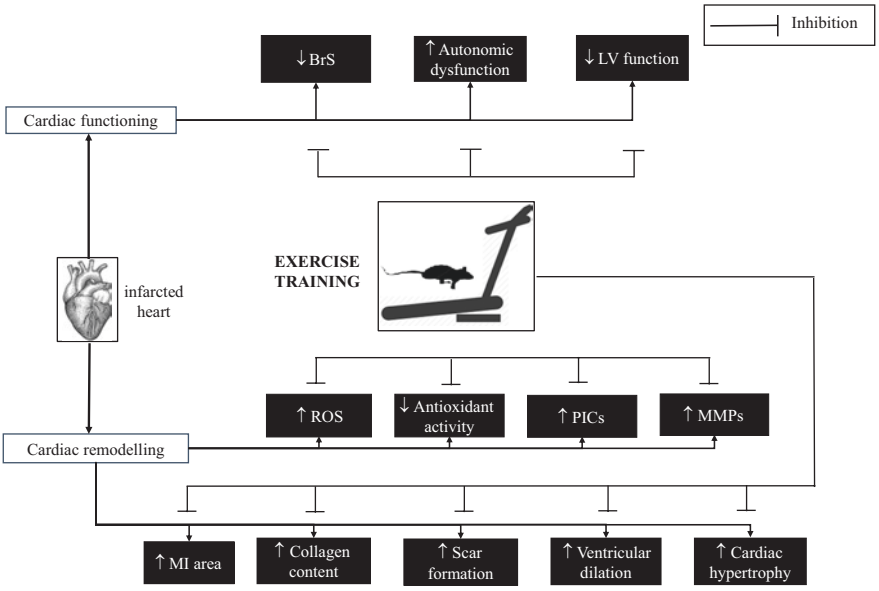

Fig. 9.2 Main effects of exercise training (ET) in experimental myocardial infarction (MI). Aerobic ET performed on a rodent-adapted treadmill is the most studied method. *BrS* baroreflex sensitivity, *LV* left ventricle, *ROS* reactive oxygen species, *PICs* proinflammatory citokynes, *MMPs* matrix metalloproteinases

cardiac remodelling are still necessary. Figure 9.2 summarizes the main positive effects of ET in experimental MI.

6 Exercise Training and MI Associated with Chronic Diseases or Conditions

MI can be associated with other pathophysiological conditions which are usually present previously to the cardiac event, acting, indeed, as a MI risk factor or as additional complication factors after MI.

6.1 Menopause

Menopause is characterized by several hormonal alterations in adult women, mainly in the estrogen levels, which declines around 60% [43]. Since estrogen is strongly associated with endothelial function, fat deposition, inhibition of vascular smooth cells growing, among others, its decrease during menopause collaborate to increase the cardiovascular risk during this period [44]. In fact, during this phase of life, women commonly show high blood pressure values, increased intima-media thickness of the carotid and femoral arteries, increased arterial stiffness, as well as impairment of flow-mediated vasodilation [45]. Therefore, menopausal women show elevated risk to suffer from MI [46, 47]. Lifestyle changes, including the practice of ET, have been strongly suggested to this population, in an attempt to mitigate the risks and comorbidities associated with menopause [48].

In this sense, some experiments have been designed to identify the impact of ET on menopausal MI rats. In the experiment of Almeida et al. [47], authors underwent ovariectomized (OVX) rats to MI and, 2 weeks later, started ET. The protocol of exercise occurred 5 days per week, during 8 weeks. Results did not demonstrate effectiveness of ET to alter MI extension. Therefore, MI extension was similar between OVX MI sedentary and trained rats. Fluorescence analyses indicated that SOD production was increased in the heart of MI rats. However, ET could prevent this alteration. Protein expression of components of the RAAS system (i.e., AT1 receptor) was increased in the heart of ovariectomized MI rats when compared to sham group. However, ET decreased the expression of these proteins, as well as increased catalase. Lastly, ET decreased collagen deposition in the left ventricle, which was high in OVX MI rats [47]. Taken together, these data indicate that ET could modulate pathways associated with cardiac remodelling. However, the absence of a morphological alteration (i.e., MI) could indicate that, to this population, ET program must be different, using, for example, a longer time of intervention.

Nevertheless, another study, which also underwent OVX MI rats to ET, showed improved BrS, cardiac autonomic control and resting bradycardia in the animals

after ET [46]. Moreover, correlations were observed between autonomic improvements (reduction of sympathetic activity and increased in vagal activity) and bradycardic response, which suggests that improvement in BrS would occur due to improvement of autonomic control [46].

6.2 Diabetes

Diabetes is related to several cardiovascular risk factors, such as: dyslipidemia, atherosclerosis, diabetic autonomic neuropathy, inflammation, increased ROS formation, impairment of flow-mediated vasodilation, among others [49, 50].

When together with cardiovascular disease, as MI, additional complications are observed in diabetic rats, such as augmented autonomic impairment represented by reduced BrS and vagal tone. Moreover, cardiac autonomic dysfunction was associated with impaired hemodynamic function and cardiorespiratory capacity, as well as increased mortality rate [51]. On the other hand, when diabetic and infarcted rats underwent ET, animals showed increased mRNA and protein expression of vascular endothelial growth factor (VEGF), as well as regularization in the elements involved in calcium handling, to quote: SERCA2, Na^{+}–Ca^{2+} exchanger, SERCA2/phospholamban ratio and phosphor-thr17-phospholamban. In addition, authors observed normalization in the hemodynamic function and in regional blood flow, as well as improvement on autonomic function [51]. Lastly, ET collaborates to reduced mortality rate in diabetic infarcted rats [52].

6.3 Obesity

Obesity is another pathology associated with cardiovascular abnormalities. Besides its effects on metabolic profile, obesity also affects heart structure and function [53]. Moreover, adipose tissue is strongly associated with elevated inflammatory and ROS markers, both elements that collaborate to elevated cardiovascular disease and development of MI [54]. In this sense, in a study designed to identify the effects of ET on obese MI rats, authors observed that, after exercise, the heart of the animals showed decreased ROS protein expression and activity, as well as increased antioxidant activity. These alterations were associated with increased survival rate [54].

7 Conclusions

Corroborating international guidelines for cardiac rehabilitation, experimental studies confirm that exercise is of great importance to optimize cardiovascular recovery after MI, and shed light on the mechanisms that may respond for cardiac alterations

after MI and for the beneficial adaptations promoted by ET. As seen in this chapter, ET acts in key mechanisms of cardiac remodelling and cardiovascular control after MI, thus contributing both to prevent or postpone harmful adaptations, and even to recover from negative alterations caused by cardiac ischemia. Translational studies regarding ET associated with MI should be performed in order to a build parallel knowledge between basic and clinical science.

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Chapter 10
Cardiac Ischemia/Reperfusion Injury: The Beneficial Effects of Exercise

Juliana Pereira Borges and Karine da Silva Verdoorn

Abstract Cardiac ischemia reperfusion injury (IRI) occurs when the myocardium is revascularized after an episode of limited or absent blood supply. Many changes, including free radical production, calcium overload, protease activation, altered membrane lipids and leukocyte activation, contribute to IRI-induced myocardium damage. Aerobic exercise is the only countermeasure against IRI that can be sustained on a regular basis in clinical practice. Interestingly, both short-term (3–5 days) and long-term (several weeks) exercise increase myocardial tolerance, reduce infarct size area and arrhythmias induced by IRI. Exercise protects the heart against IRI in a biphasic manner. The early phase of cardioprotection occurs between 30 min and 3 h following an acute exercise bout, whilst the late phase is achieved within 24 h after the exercise bout and persists for several days. As for the exercise intensity, although controversial data exists, it is feasible that the amount of cardioprotection is proportional to exercise intensity and only achieved above a critical threshold. It is known that aerobic exercise produces a cardioprotective phenotype, however the mechanisms responsible for this phenomenon remain unclear. Apparently, aerobic exercise-induced preconditioning is dependent on several factors that work together to protect the heart. Altered nitric oxide (NO) signaling, increased levels of heat shock proteins (HSPs), enhanced function of ATP-sensitive potassium channels, increased activation of opioids system, and enhanced antioxidant capacity may contribute to exercise-induced cardioprotection. Much has been discovered from animal models involving exercise-induced cardioprotection against cardiac IRI, however translating these findings to clinical practice still represents the major challenge in this field.

Keywords Heart • Ischemia/Reperfusion injury • Signaling • Myocardial

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1 Introduction

Coronary artery disease (CAD) continues to be one of the major causes of debilitating disease and death worldwide, making up more than half of all cardiovascular events in men and women <75 years of age. In United States, on average, every 34 s an individual has a coronary event, and approximately every 1 min 24 s, someone will die of one [1].

Several CAD complications (e.g. heart failure and myocardial infarct) are primarily caused by IRI damages on the myocardium [2]. Considering that duration and magnitude of the ischemic insult predict tissue injury level or death, restoring local blood supply is crucial to reduce infarct size area [3]. Despite this, reperfusion through thrombolytic therapies or percutaneous coronary intervention may induce further damages ranging from functional impairments to cell death [4].

In light of the severity of IRI clinical outcomes, several researchers around the world have focused on studying new strategies to protect the heart against IRI. Although effective strategies protecting the heart have been discovered, translating these findings to clinical setting has been challenging [2].

In this scenario, physical exercise plays a key role as it may be the unique cardioprotective strategy that can be easily applied regularly in clinical practice and actually improve patient's outcome. Indeed, several studies have demonstrated that exercise not only reduces cardiovascular risk factors, such as hypertension and obesity, but also promotes cardioprotection against IRI through a direct effect on the myocardium [5, 6]. Previous data have shown that a 20-week exercise training in treadmill significantly improved the intraventricular pressure and reduced the infarct size by 25% of rats submitted to 1 h of ischemia followed by 2 h of reperfusion [5]. Interestingly, it has been demonstrated that performing as few as three to five aerobic exercise sessions prior to an ischemic event are also sufficient to promote increment in left ventricle function [7, 8], reduce ventricular arrhythmias [9] and infarct size area [10–12] upon reperfusion.

Although it is clear that aerobic exercise produces a cardioprotective phenotype, the mechanisms responsible for this phenomenon remain unclear. It is believed that these mechanisms are multifactorial and include: (1) changes in coronary arteries (i.e., increased collateral circulation) [13, 14], (2) altered NO signaling [15–18], (3) increased levels of HSPs [19–21], (4) amplified myocardial cyclooxygenase-2 (COX-2) activity [22], (5) elevated endoplasmic reticulum stress proteins [23, 24], (6) enhanced function of sarcolemmal and/or mitochondrial ATP-sensitive potassium channels (K_{ATP}) [25, 26], (7) increased activation of opioids system [10, 11], and/or (8) increased cytosolic/mitochondrial antioxidant capacity [12, 27, 28].

The following sections describe the pathophysiology of cardiac IRI and how short and long-term exercise could benefit the heart by attenuating damages induced by IRI.

2 Ischemia/Reperfusion Injury Pathophysiology

Ischemia consists of a lack of blood supply to the tissue. It can result due to augmented tissue metabolism (as in exercise) without accompanying blood flow increase or due flow obstruction resultant from vasoconstriction, thrombosis or embolism. These blood supply/demand imbalances cause varying degrees of tissue insult depending on intensity and duration of ischemia and intrinsic tissue metabolism. Under normal circumstances cardiac myocyte metabolism is predominantly aerobic (~95%) and therefore these cells possess a high oxygen demand. Of the great amount of produced ATP, about 2/3 is used by the contractile apparatus to afford contraction and 1/3 by active ion transport proteins (mainly SERCA and Na^++K^+-ATPase) to maintain ion balance [29, 30]. Upon ischemia, these cell processes, and consequently cell function, will be greatly affected.

Inadequate oxygen supply rapidly decreases mitochondrial ATP production and depletes the cells high energy phosphates (mainly creatine phosphate). The underperfused cardiomyocytes switch from oxidative to anaerobic metabolism and immediately downregulate contraction adapting its mechanical work to its energy supply. Underlying mechanisms that trigger these adaptations involve depletion of creatine phosphate pool, accumulation of lactate and intracellular acidosis [3, 31]. Perfusion-contraction match decreases energy consumption and oxygen demand. These work and metabolic changes consist of a short-term (about 15 min of severe ischemia) defense mechanism to postpone irreversible injury and avoid cell death.

Anaerobic glycolytic metabolism is not only far from sufficient to sustain contraction and ionic balance, but it also has a biphasic nature. While at the onset of ischemia glycolytic activity is stimulated, with prolonged or severe ischemia it decreases because of impaired glucose delivery, glycogen depletion and accumulation of inhibitory metabolites (its end products pyruvate and reduced nicotinamide adenine nucleotide – $NADH_2$).

The ATP-depleted cardiomyocytes have compromised ATPase activity causing ionic imbalance. Reduced Na^++K^+-ATPase activity increases intracellular Na^+ and is unable to impede net K^+ efflux due to the opening of the K_{ATP} channels (gated by intracellular ATP/ADP). Accumulated hydrogen ions (H^+), produced during anaerobic glycolysis, are exchanged for Na^+ by the Na^+/H^+ exchanger (NHE). This counteracts further intracellular pH reduction but adds even more Na^+ to the intracellular pool contributing to cell swelling. Calcium efflux via plasmatic membrane Ca^{2+} ATPase (PMCA) and reuptake by the endoplasmic reticulum Ca^{2+} ATPase (SERCA) are impaired and cytosolic Ca^{2+} overload ensues (see Fig. 10.1 and video 10.1).

As ischemia advances, mitochondria accumulates ischemic damage (cardiolipin and cytochrome c lose into the cytosol and oxidative phosphorylation becomes uncoupled). These damages are mediated by mitochondria themselves, that in the presence of residual oxygen present a reduced flow of electron transport activity and consequent production of reactive oxygen species (ROS). Studies have demonstrated that blocking mitochondria electron transport chain activity (with amobarbital or rotenone, both block complex I reversibly) immediately prior to ischemia prevents

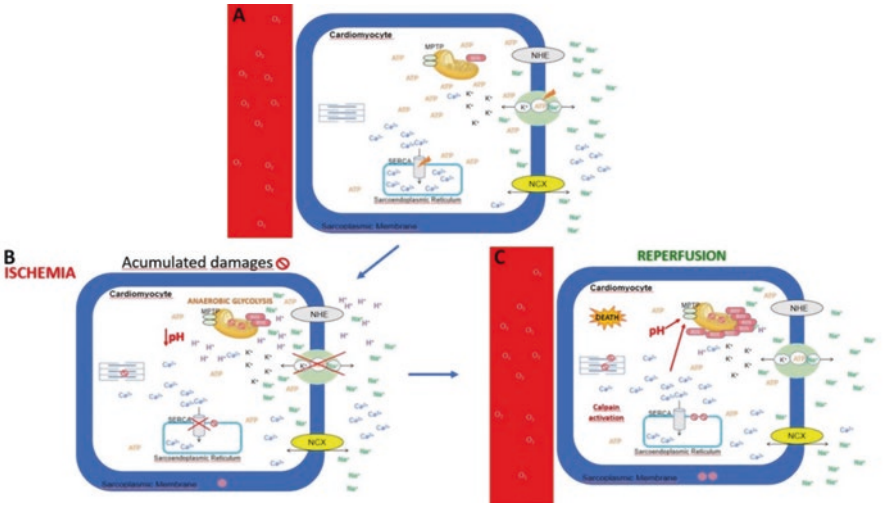

Fig. 10.1 Pathophysiology of ischemia reperfusion injury. (**a**) Normal cardiac myocyte cell function and ion distribution. (**b**) Ischemia-induced cell damage and ion balance disruption. (**c**) Reperfusion-induced aggravated cell damage. ROS (reactive oxygen species); MPTP (mitochondrial permeability transition pore); SERCA (sarcoendoplasmic reticulum Ca^{2+} ATPase); NHE (sodium/hydrogen exchanger); NCX (sodium/calcium exchanger)

mitochondrial damages and preserves its respiratory function and cardiac myocyte viability [32, 33], confirming mitochondrial damage occurs during the ischemic period. During ischemia, low intracellular pH precludes the opening of mitochondrial permeability transition pore (MPTP) [34, 35], which would be greatly favored by the accumulated mitochondrial injuries.

Although restitution of blood flow should halt and rescue cells form progressive ischemic injury it actually triggers an adverse cascade of events and may precipitate death of more severely damaged cardiomyocytes. Reperfusion restores oxygen and nutrient supply, necessary for aerobic ATP synthesis, unfortunately accumulated mitochondrial ischemic injuries impair electron transport chain activity and cause accelerated and profuse ROS formation. Reperfusion-induced oxidative stress aggravates cardiomyocyte injuries since all cellular components are affected by ROS. The sarcoplasmic membrane has its fluidity and permeability altered, sarcoplasmic reticulum becomes stressed, enzymes dysfunctional and NO (important protective signaling molecule) bioavailability is reduced. Reflow washes out extruded ions and metabolites, and brings with it cells of the immune system. Because extracellular and intracellular pH are normalized by removal of accumulated H^+ and lactate, MPTP is activated and mitochondrial membrane potential dissipated. MPTPs are located in the inner mitochondrial membrane and form nonselective pores that, when opened, cause mitochondrial membrane depolarization and can result in water entry into the matrix, swelling and outer mitochondrial membrane rupture. This culminates with mitochondrial proapoptotic molecules

release and cell death via caspase-dependent and independent mechanisms [36] (Fig. 10.1 and Video 1).

Intracellular Ca^{2+} overload is exacerbated during reperfusion, because SERCA and PMCA are still inactive and now cytosolic sodium is extruded in exchange for calcium by the Na^{+}/Ca^{2+} exchanger (NCX). To prevent lethal increase in cytosolic calcium, mitochondria import Ca^{2+} into its matrix (via a Ca^{2+} uniporter), paradoxically if in excess mitochondrial Ca^{2+} activates MPTP opening. Free intracellular calcium also activates proteases (calpains), which damage myofibrils, degrade cytoskeletal, endoplasmic reticulum and mitochondrial proteins, and trigger intracellular signaling pathways (Ca^{2+}/calmodulin-dependent protein kinases) that may conduct to cell death. Furthermore, inflammatory response can be elicited with reperfusion, due to ischemic cardiomyocytes release of proteins (damage-associated molecular patterns - DAMP) and expression of toll-like receptors (TLR) causing leucocyte recruitment [37]. Activated neutrophils secrete ROS and leukotrienes and reduce endothelial NO availability, leading to microvascular damage (vasomotor dysfunction, increased permeability and angiogenesis) and possibly new vascular occlusion (due to endothelial cell swelling and neutrophil or platelet plugging), which hamper complete coronary flow restoration or lead to subsequent new ischemic episode [38–40].

Ischemia and reperfusion-induced cardiomyocyte homeostatic derangements may culminate with cell death. Major patterns of cell death are necrosis (also called oncosis), apoptosis and autophagy. Necrosis is defined as an uncontrolled process, characterized by organelle and cell swelling (therefore the term oncosis – cell death by oncotic pressure), plasmatic membrane rupture and intracellular content leakage, giving rise to inflammation and scar tissue formation. Recent evidence of molecular pathways activating necrosis outdated the uncontrolled nature of this pattern of cell death, giving rise to the term necroptosis. Although its pathway shares upstream signaling elements with apoptosis (such as TNF), it is a caspase independent and morphologically distinct form of cell death [41]. Differently, apoptosis is a genetically programmed, energy-dependent (ATP consuming) process that involves nuclear condensation, DNA fragmentation, phagocytosis of apoptotic bodies in the absence of inflammatory reaction. Specific extracellular (involving activation of Fas and TNFα receptors) and intracellular (mitochondria mediated) pathways regulate the apoptotic process mediated by caspase proteases. Autophagy, the third mechanism of cell death, is actually a housekeeping and cell survival mechanism, whereby cell components (including damaged or unnecessary organelles) are captured degraded and recycled through vesicles that fuse to lysosomes. It is a process regulated by autophagy-related genes (Atg), however under pathological conditions uncontrolled autophagy may lead to cell death [42, 43].

All types of myocardial cell death have been identified after IRI, although relative contribution of each type, as well as the moment of its trigger (during ischemia or reperfusion), remain obscure issues [44]. Experimental evidence points to initial cell death during prolonged ischemia, with reperfusion greatly exacerbating the magnitude of cardiomyocyte loss [45]. Even after periods of non-lethal ischemia, reperfusion injury triggers cell death. Importantly, reperfusion can elicit two waves

of cell death: the first one in the acute initial phase of reperfusion injury and the second one in a more chronic period (days) post-reperfusion. While the mechanisms responsible for the acute phase of reperfusion cell death were described previously (excess ROS, Ca^{2+} overload and MPTP activation), the mechanisms in post-reperfusion cell death include reminiscent irreversibly injured myocytes (once cell death can take several hours to completion), regions of myocardial no-reflow and progressive inflammatory reaction [46].

Due to limited capacity of cardiac myocytes for proliferation, even low levels of cell death can result in myocardial functional impairment. Identification and quantification of the different types of cell death may help to understand consequent myocardial structural composition and the resultant functional behavior.

2.1 Levels of Post-reperfusion Myocardial Dysfunction

In the previous section a spectrum of cell derangements elicited by ischemia and reperfusion was described. The level of myocardial function impairment that will ensue depends on duration of ischemia, extent of committed area and pattern of reperfusion.

Early and prompt restitution of coronary flow after an ischemic episode may result in reperfusion arrhythmia. It occurs immediately with the onset of reperfusion and is explained by large intracellular calcium oscillations. Short periods of ischemia followed by sudden reperfusion allow for cytosolic calcium overload and excess recycling upon restitution of ATP level. Repetitive extreme calcium oscillations result in transient ionic inward current and delayed after depolarizations [47]. This phenomenon is most likely to occur in the experimental setting where abrupt reversion of coronary occlusion takes place. To date, only preliminary antiarrhythmic action of selective inhibitors of NCX have been published [48].

A second level of myocardial dysfunction despite fully restored coronary flow is stunning. In the stunned heart revascularization is complete and successfully achieved in the absence of permanent damage, notwithstanding myocardial contractile function remains depressed. Full mechanical recovery can take hours, days or even weeks to be accomplished [49, 50]. The reduced mechanical efficiency is explained by an intracellular functional remodeling resulting in decreased responsiveness of the contractile filaments to Ca^{2+}. It is an adaptive response triggered by Ca^{2+} overload and ROS damages to the contractile apparatus [51, 52]. It has been proposed that full myocardial recovery is accomplished after the damaged contractile proteins have been resynthesized [52].

Repetitive ischemia and stunning episodes can lead to a condition called hibernation, also characterized by myocardial hypo-contractility. In this condition, depressed contractile function due to limited blood perfusion prevails. It is a vascular dysfunction in the absence of cell damage enabling cardiomyocytes to resume contractile activity when revascularization takes place. Nevertheless, with prolonged chronic hibernation cardiomyocyte may atrophy and dye. Although the two conditions, stunning and hibernation, can coexist, the first one is a hypo-contractile

period caused by full reperfusion and hibernation refers to hypo-contractile but viable myocardium under limited blood flow [53].

Periods of prolonged ischemia followed by revascularization elicit the most severe level of IRI. Cardiomyocytes become irreversibly damaged and substantial cell death can lead to tissue remodeling, progressive cardiac dysfunction and chronic ischemic cardiomyopathy.

3 Cardioprotection Against Ischemia/Reperfusion Injury

Due to this major clinical problem, investments in therapeutic approaches to treat IRI have been considered of importance in the research field [54]. On this matter, Murry et al. [55] showed infarct size reduction after submitting anesthetized dogs to 4 periods of 5 min coronary artery occlusion interspaced by 5 min-periods of reperfusion before the onset of a 40 min sustained coronary occlusion. This phenomenon, known as ischemic preconditioning, is a method whereby repeated brief ischemia episodes confer cardioprotection against a subsequent longer ischemic insult (as represented in Fig. 10.2A). It has been speculated that the preconditioning stimuli triggers a signaling cascade of intracellular events and thus create a memory effect that attenuates IRI. Among the putative mediators of this cardioprotection signal are bradykinin, norepinephrine, adenosine, inhibitory guanine nucleotide binding proteins, free radicals, opioids, protein kinase C (PKC), sarcolemmal and mitochondrial K_{ATP} channels [56]. Ischemic preconditioning is a biphasic phenomenon with two distinct windows of cardioprotection – the first one occurs within few minutes after the ischemic insult and persists for only 1–2 h, while the second one starts after 12–24 h of IRI and lasts for 48–72 h [57].

The initial observation of ischemic preconditioning has encouraged innumerous scientists to investigate other possible cardioprotective strategies [4, 54]. The major strategies developed so far are briefly presented below:

- Ischemic post-conditioning: in contrast to unimpeded reperfusion, slowly bringing the heart out of ischemia salvages myocardial cells (Fig. 10.2B). This intermittent reperfusion interspaced with brief periods of myocardial ischemia after a prolonged ischemic insult has been reported to attenuate IRI by reducing the infarct size [58, 59].
- Remote ischemic preconditioning: it was first described in 1993 [60] that brief episodes of ischemia in circumflex branch reduced the infarct size induced by 1 h of sustained left anterior descending coronary artery occlusion and 4.5 h of reflow. In other words, brief episodes of ischemia in one vascular bed protect remote myocardium from subsequent sustained coronary artery occlusion of another vascular bed. Unfortunately, the difficulty of accessing the heart's arteries may hinder the use of such strategy in most clinical interventions. On the other hand, cardioprotection has also been achieved by applying brief periods of ischemia to tissues or organs other than the heart, which could be easier for clinical application [61].

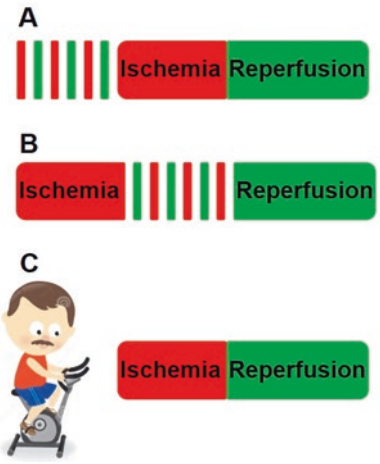

Fig. 10.2 Representative diagrams of strategies developed to counteract ischemia reperfusion injury. (**a**) Ischemic preconditioning: repeated short episodes of ischemia followed by a subsequent prolonged ischemic insult. (**b**) Ischemic post-conditioning: intermittent reperfusion interspaced with brief periods of myocardial ischemia after a prolonged ischemic insult. (**c**) Exercise preconditioning: aerobic exercise performed before ischemia reperfusion protects myocardium

- Pharmacological preconditioning: volatile anesthetics used for analgesia during surgeries have been found to improve tolerance to myocardial ischemia and post-ischemic recovery [56]. Since then, these agents have been extensively used in patients undergoing surgery, especially when ischemia and reperfusion periods are planned [56].
- Exercise preconditioning: previous studies indicate that aerobic exercise prior to ischemia reperfusion improves myocardial tolerance to IRI [62] (Fig. 10.2C).

Repetitive exposure of the myocardium to ischemia eventually leads to cardiac dysfunction, while prolonged pharmacotherapy leads to tissue desensitization [63]. Aerobic exercise therapy, by contrast, is pragmatic in that it is cost effective and sustainable for extended treatment. Therefore, exercise preconditioning is considered the only viable therapy that may provide long-term protection against IRI from a scientific perspective [64]. Several studies have demonstrated that exercise not only reduces cardiovascular risk factors, such as hypertension and obesity, but also promotes a direct effect on the myocardium protecting against IRI-induced arrhythmias [9, 65, 66], myocardial stunning [67–69], and myocardial infarction [10, 12, 70].

4 Exercise-Induced Cardioprotection Against Ischemia/Reperfusion Injury

Cardioprotection afforded by exercise represents an increasing field of research. As seen in Fig. 10.3, the number of citations found on pubmed using the terms “exercise” AND “cardioprotection” has risen significantly since 1987.

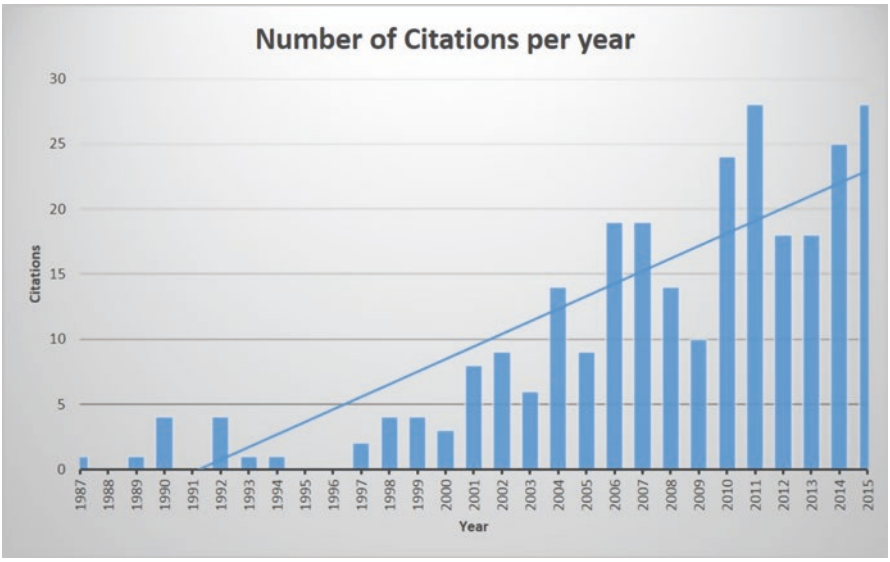

Fig. 10.3 Number of citations per year since 1987 found on pubmed using the terms "exercise" and "cardioprotection"

The notion that exercise training is cardioprotective against IRI is well established in animal models [71, 72] and human epidemiological studies [73–75]. In fact, physically active individuals are less susceptible to cardiovascular events and have a greater survival rate following a heart attack in comparison with their inactive counterparts [71, 73]. As for experimental settings, well-controlled animal studies beginning in the late 1970s have provided convincing evidence that long and short-term aerobic exercise elicit cardioprotection against coronary artery occlusion [5–8, 10–12, 76, 77].

Data on the cardioprotective effect of long-term (weeks to months) and short-term exercise (1–5 days) against IRI as well as its time course and the influence of exercise intensity are presented in the next sections.

4.1 Long-Term Exercise

The study of McElroy et al. [76] was one of the first to demonstrate that regular bouts of exercise training were capable to confer cardioprotection. In their study, rats were submitted to swimming 1 h/day, 5 days/week for 5 weeks and 24 h after the end of the training the rats underwent left coronary occlusion. Following 48 h after surgery, the authors observed a reduction in infarct size by 30% of exercised rats in comparison to controls (21.5 ± 1.9% vs 31.3 ± 2.6%; $P < 0.05$). Similarly, Brown et al. [5] observed an enhanced cardiac function and 25% reduction in the infarcted area among animals trained for 20-week after 1 h of ischemia and

2 h of reperfusion. In addition, Powers et al. [6] showed that, compared with untrained, exercised animals kept a higher systolic blood pressure throughout regional ischemia and reperfusion protocol after a 10-week endurance exercise training program.

Apart from these data, several other studies have underpinned that long-term exercise benefits the heart against all three levels of IRI [22, 67, 68, 78–81]. However, a criticism exists - most studies applied ischemia within 48 h after the last exercise session [6, 67, 78, 80, 81] and acute bouts of exercise have been demonstrated to protect the heart against IRI for at least 48 h [12]. Therefore, this choice of interval between the last exercise bout and ischemia may have jeopardized the assessment of cardioprotection afforded exclusively by chronic training.

4.2 Short-Term Exercise

After the first evidence in the late 1970s that exercise training for several weeks provided cardioprotection against coronary occlusion [76], investigating the cardioprotective effect of acute exercise was just a matter of time. Discovering if an intrinsic cardioprotective response could be rapidly acquired had crucial impact to understand the underlying mechanism and the exercise "dosage" required for optimum protection [8].

In this sense, Locke et al. [82], using the retrograde perfused Langendorff heart preparation, observed that rats exercising for only three consecutive days also had improved post-ischemic developed pressure following IRI. However, just a single bout did not change any measure of post-ischemic cardiac function. Data from Yamashita et al. [12] partially concur with the results found by Locke et al. In this study, untrained rats and those exercised for a single bout of 30 min at 30 m/min were submitted to regional ischemia and reperfusion. The authors have demonstrated that the rate–pressure product showed no significant difference among the groups after 20 min of ischemia or 30 min after reperfusion. However, exercised rats exhibited a marked decrease relative to control in the size of myocardial infarct.

On the other hand, Taylor et al. [8] after submitting 1 and 3 days trained rats to IRI found that cardiac output of both groups was equally superior than untrained rats. Several differences between the adopted experimental protocols in these studies, especially regarding the measured outcomes, ischemia/reperfusion protocol or exercise duration and intensity, could account for the discrepancy seen in the results. Nonetheless, although some authors advocate the opposite [82], most studies argue that a single exercise session would be enough to provide some degree of cardioprotection against IRI [8, 12]. It is clearer, however, that at least three consecutive exercise sessions benefit the heart against IRI, as data in this sense accumulates in the literature [7, 10, 11, 77, 83].

The question that remains to be answered is whether short-term exercise provide cardioprotection against IRI just as effective as long-term exercise or not. Despite the lack of interventional settings focused in this specific matter, previous reviews

claim that exercise-induced cardioprotection following few exercise sessions is similar to long-term physical training [24, 62]. Given that exercise-induced cardioprotection is a multifactorial process, it is tempting to assume that it involves different mediators and magnitude according to the exercise protocol duration. Clarifying this issue would be important considering that the potential mechanisms involved in this response are still largely debated.

4.3 Exercise Training Intensity

When it comes to exercise intensity, the first question we ask is how much exercise is needed to protect the heart. Is there a minimum amount of exercise to achieve cardioprotection? Many researchers have been trying to answer this question, as the dose-response impact of aerobic exercise intensity on cardioprotection is extremely important. And the truth is that although findings from several studies provide insight into this matter [24], definitive answers remain unknown.

Before reviewing existing data on the effect of exercise intensity over cardioprotection, it is important to revisit a concept of exercise prescription. There are two different methodologies to deal with aerobic exercise intensity: continuous or interval exercise. Continuous exercise consists of maintaining submaximal power output and VO_2 constant throughout the entire session, whilst interval exercise alternates periods of greater and lower intensity within an exercise session [84].

In one of the first investigations into the role of long-term exercise intensity in providing cardioprotection [85], rats were treadmill trained for 11–16 week at low intensity (20 m/min, 0% grade, 60 min/day), moderate (30 m/min, 5% grade, 60 min/day) or intense (10 bouts of alternating 2-min runs at 16 and 60 m/min, 5% grade). After submitted to 25 min of global ischemia, all trained groups presented significantly greater post-ischemic cardiac output and work compared to sedentary rats. However, as intensity increased the greater was myocardial recovery.

Interestingly, a previous study [67] exercised rats daily for 6 week at low (20 m/min, 0% grade, 60 min/day) or high intensity (5 bouts of alternating 1-min runs at 75 and 20 m/min, 15% grade, 10 min/day). The high-intensity protocol improved myocardial functional recovery following 20 min of global ischemia in an isolated Langendorff perfusion model, but the low-intensity program did not. The same result was found by Starnes et al. [81], who showed that exercise training for 16 week, 5 days/week 40 min/day below 55–60% VO_{2max} did not achieve protection against IRI. Unfortunately, the earlier studies did not provide information regarding exercise intensity used in terms of VO_{2max}. Regardless, it is feasible that there may be an exercise intensity threshold above which cardioprotection is achieved and that the amount of cardioprotection is proportional to exercise intensity [24, 86].

Controversially, Lennon et al. [87] concluded that both moderate- (i.e., 60 min/day at 50% VO_{2max}) and relatively high-intensity exercise (i.e., 60 min/day at 70% VO_{2max}) performed during three consecutive days appear to be equally protective against IR-induced myocardial stunning. The discrepancy in results may be due to

the use of different exercise protocols in regards to: a) exercise training duration (long or short-term) that possibly alters the underlying mechanisms for cardioprotection; and b) methodology for imposing intensity (continuous or interval) that could interfere in the amount of cardioprotection afforded. Besides investigating these issues, it would be interesting to assess the effects of exercise intensity following different levels of IRI other than myocardial stunning, as all previous data in this matter were found after applying ischemia up to 25 min [67, 81, 85, 87].

Recently, to overload the physiological system and stimulate greater adaptations due to an increased shear stress, high intensity interval training (HIIT) has been increasingly prescribed to patients with cardiovascular diseases. Indeed, well-controlled clinical studies in patients with heart failure indicated that interval exercise affords greater benefits in mitochondrial biogenesis, insulin sensitivity [88], and body fat reduction [89] than continuous exercise training. However, the potential role of HIIT in providing protection against IRI is still little understood.

Importantly, regardless of the choice for interval or continuous exercise, studies investigating the influence of training intensity on cardioprotection should use exercise bouts with equal volume (understood as the interaction between training intensity, duration and frequency) [90]. Otherwise, exercise sessions performed with a higher intensity might have a greater volume, which introduces bias in such experiments designed to determine whether a given outcome was exclusively produced by exercise intensity or not. Unfortunately, exercise induced-cardioprotection has never been studied in exercise sessions with different intensities but equal volume.

4.4 Time Course for Exercise Cardioprotection

Similar to the ischemic preconditioning phenomenon, an interesting study revealed that exercise-induced cardioprotection is biphasic [12]. In this study, rats were exercised for a single bout for 30 min at 30 m/min and then underwent regional cardiac ischemia/reperfusion (20 min/48 h) at 0.5, 3, 24, 36, 48, 60, and 72 h after exercise. The authors observed that the size of myocardial infarct after IRI in rats at 3, 24 and 72 h after exercise was similar to untrained rats. However, rats subjected to ischemia at 0.5, 36, 48, and 60 h after the exercise session exhibited a significantly decrease in infarcted area in comparison to control rats. Thus, the first phase of cardioprotection is believed to start rapidly following an acute exercise bout (i.e., 0.5 h after exercise). Nonetheless, this early cardioprotection is quickly lost within 3 h post-exercise. The second or late phase of exercise-induced cardioprotection is acquired within 24 h after the exercise bout and persists for several days.

As important as the time required to achieve cardioprotection is for how long after detraining the acquired phenotype is maintained. In Lennon's study, short-term exercised animals (3 days of 60 min/day at 30 m/min) kept a higher cardiac work after global ischemia/reperfusion (20.5 min/30 min) at 1, 3, and 9 days post-exercise. The exercise-induced cardioprotection vanished by 18 days after exercise cessation [20]. As for long-term exercise, cardioprotection against IRI seems to persist for a

little longer. Esposito et al. have demonstrated that, although some cardioprotection was kept, 10-week trained rats had higher infarct size after 4 weeks of detraining [86].

4.5 Resistance Exercise Preconditioning

It is well established that resistance training induces cardiac hypertrophy due to pressure overload during training [91]. Although there is a notion that concentric and eccentric cardiac hypertrophy occurs in response to resistance and endurance training, respectively; more recent evidence cast some doubts over this proposal [92, 93]. In addition, previous data reported benefits of resistance exercise on cardiac performance in patients with heart failure [94]. Even though it is not clear whether significant adaptation in cardiac structure is possible in individuals undergoing resistance exercise training, investigating the impact of this type of training on IRI is crucial.

Although convincing evidence exists that short and long-term aerobic exercise induce cardioprotection against IRI, the role of resistance training in protecting the heart in this sense is little understood. In this matter, only two studies from the same group have been developed so far [91, 95]. In the first one [95], rats were exercised in a squat-training apparatus (12 repetitions/set, 4 sets/day and 5 days/wk. for 12 weeks) and after the training cessation underwent transient regional ischemia of left anterior descending coronary artery (40 min) followed by 80 min of reperfusion. The authors observed that diastolic pressure and infarct size were smaller in trained rats whereas coronary flow and developed pressure were higher in trained than untrained rats during and after the cardiac insult.

Interestingly, the second study included identical protocol, except for the shorter resistance training duration, but found different results. Four weeks of resistance training did not significantly change the infarct size, apoptosis rate and myocardial tolerance against IRI. Therefore, the duration of resistance training seems to play a key role in inducing cardioprotection. This may be due to a required longer period to adaptations after resistance training to occur. Nevertheless, a precise conclusion about this issue needs more investigations.

5 Mechanisms Involved in Cardioprotection Against Ischemia/Reperfusion Injury

As described earlier, many changes that include calcium overload, free radical production, altered membrane lipids, protease activation, and leukocyte activation, contribute to IRI-induced myocyte damage. In theory, any physiological effect elicited by exercise that influences one or more of these events and attenuates myocyte damage, acts as a mechanism of cardioprotection. In this sense, exercise induces cardioprotection by multifactorial processes.

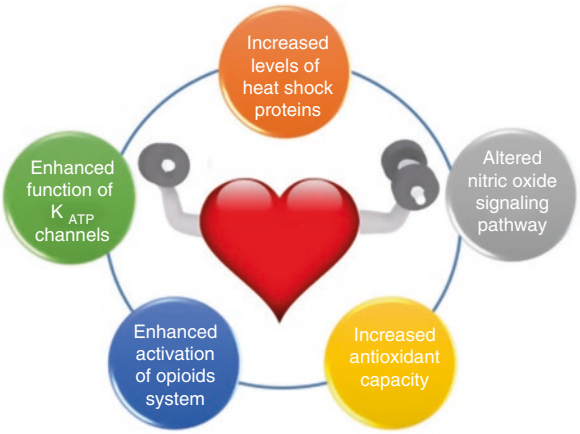

Fig. 10.4 Candidate mechanisms for exercise-induced cardioprotection

The reported major mechanisms of exercise-induced cardioprotection include (Fig. 10.4): (1) increased antioxidant capacity, (2) increased levels of HSPs, (3) altered NO signaling pathway, (4) enhanced function of K_{ATP} channels, and (5) increased activation of the opioids system [54]. Other possible underlying mechanisms such as: adaptations in coronary arteries (increased arteriolar diameter and density), elevated endoplasmic reticulum stress proteins, and amplified myocardial COX-2 activity are not manifested after an acute bout of exercise [23, 24, 62] and, although can contribute to chronic exercise cardioprotection, are not considered of critical importance.

When the body is under stress, such as hypoxia, hyperthermia, acidosis and ischemia, the synthesis of several important proteins that maintain homeostasis is compromised [23]. Our organism responds to these stresses by synthetizing proteins termed HSPs that help maintaining homeostasis [71].

HSPs are classified into groups according to its molecular weight. Although many of them are related to cardioprotection, the HSP70 family deserves attention [23], especially HSP73 and 72. HSP73 is constitutively synthetized in all cells, and its level increase slightly after a stressful condition. Conversely, HSP72 is found only after stressful events, particularly IRI [96].

Not surprisingly, HSP70 is significantly increased in hearts of animals submitted to a single bout of aerobic exercise of at least 40 min [97] due to increased body temperature. In theory, elevated cellular levels of HSP72 can protect the myocardium against IRI by repairing unfolded proteins and/or by stabilizing the function of endoplasmic reticulum via HSP70-related autophagy [98].

Indeed, Locke et al. [82] have demonstrated that three consecutive days of exercise or heat stress increased HSP72 content and promoted greater myocardial recovery after IRI than controls and rats exercised only once. Others support the hypothesis that cardioprotection induced by long-term [19, 21, 86] and short-term exercise [7] can be mediated by exercise-induced increase in HSP72 levels. Controversially, many studies compared animals submitted to exercise in a cold environment and

room temperature and found that, regardless of the amount of HSP72, cardioprotection was similar between groups [8, 70, 99]. In addition, Starnes et al. [81] stated that elevated myocardial HSP70 does not necessarily imply in improved protection against myocardial dysfunction, as they observed that exercise training increased HSP70 2.7-fold, but did not provide enhanced protection following 20 min of ischemia. Therefore, although an increased HSP72 level may confer protection, this does not seem to be a prerequisite for exercise-induced cardioprotection [20].

5.1 Altered Nitric Oxide Signaling

The observations that exercise cardioprotective effect lasts longer than the return of antioxidant enzymes and HSP72 to pre-exercise levels suggested that another cytoprotective molecule was responsible for providing protection during IRI [20]. This conclusion has stimulated studies focused in the involvement of NO in exercise-induced cardioprotection given that exercise induces an increase in NO that lasts for at least 1 week after the end of training [100, 101].

Exercise causes high vascular wall shear stress throughout the body, which in turn increases the expression and activity of eNOS [102] and iNOS [16], resulting in an increase in NO and its metabolites (nitrate and nitrosothiols) [100, 101]. The exact cardioprotective effects attributed to NO and its metabolites during IRI is a subject of much debate; however, hypothesized mechanisms include reduction in ROS, augmented coronary flow due to increased vasodilation, inhibition of calcium influx into myocytes, antagonism of β-adrenergic stimulation, reduction in cardiac oxygen consumption, and actions on sarcolemmal K_{ATP} channels through cGMP-PKG signaling [23]. Together, these changes could protect cardiac myocytes against IRI. Babai et al. [16], for instance, showed that exercise reduced the severity of arrhythmias during IRI that was abolished after using a nitric oxide inhibitor. Although evidence of the involvement of NO in the cardioprotective effect of exercise accumulates in literature [15, 17, 18], it should be mentioned that controversial data exist. Eventually, exercise may produce excessive NO that could react with anion superoxide, forming peroxynitrite, which is cytotoxic [103]. In addition, Taylor et al. [104] reported that exercise induced cardioprotection against IRI, even when NO production was blocked. Therefore, although the putative importance of NO signaling in providing cardioprotetion cannot be ruled out, it should be better addressed.

5.2 Antioxidant Capacity

Imbalance between oxidation-reduction reactions and cellular antioxidant defense mechanisms is called oxidative stress. ROS and other oxidants (including NO) are generated as by-products and intermediates of normal cell metabolism, these

chemicals also act as important molecules in signal transduction and gene regulation. Therefore, oxidative stress involves not only direct damages to cellular components (proteins, lipids, DNA and others) but also altered signaling pathways and control mechanisms [3, 105, 106].

As one of the hallmarks of IRI is the huge ROS production, especially at reperfusion, exercise-induced adaptations of the myocardial antioxidant buffering system have been widely investigated. Superoxide dismutases (SOD) are molecules that promote dismutation of the superoxide radical ($^{\cdot}O_2^-$) forming hydrogen peroxide (H_2O_2) and oxygen. Although not a free radical, H_2O_2 is also a potentially cytotoxic oxygen-derived molecule, that can be reduced by the enzyme glutathione peroxidase (GPx) or converted into oxygen and water by catalase [107]. Strong evidence associate increased activity of the mitochondrial form of SOD (manganese SOD - MnSOD) to exercise-induced cardioprotection [12, 27, 99]. Importantly, French et al. [27] showed that the exercise-induced increase in MnSOD activity attenuated IR-induced oxidative modification of Ca^{2+}-handling proteins and resulted in decreased calpain activation and ultimately cardiomyocyte death. The link between increased MnSOD activity and attenuated calpain activation was confirmed using MnSOD antisense oligonucleotide treatment. With the knockdown of MnSOD, protection against calpain activation was abolished and the cardioprotective effect lost [27].

Exercise-induced modulation of other antioxidants (such as catalase and glutathione peroxidase) and synergistic action of these with different adaptations (stimulation of heat shock proteins, for instance) can also be involved in the cardioprotective response [107, 108]. In addition, exercise variables as intensity and time of detraining should be considered when evaluating antioxidants involvement in cardioprotection, since each bout of exercise benefits the myocardium for a limited period of time [12, 20, 86].

5.3 Sarcolemmal and/or Mitochondrial ATP-Sensitive Potassium Channels

Activity of K_{ATP} channels, highly expressed in the sarcolemma and mitochondria, is governed by intracellular nucleotides (ATP and ADP) concentration. K_{ATP} channels remain closed with stable ATP levels; however, metabolic stress-induced (e.g. hypoxia or ischemia) ATP reduction triggers the channel opening. Cardiomyocytes K+ efflux hyperpolarizes the cardiac cells and decreases the number of action potentials. This would limit Ca2+ entrance through L-type channels and prevent intracellular Ca2+ overload and the MPTP from opening [26, 109]. As a result, cardiac metabolic demand and electron transport chain activity decrease, thereby preventing ROS production and necrotic cell death. Therefore, K_{ATP} channels are thought to function as sensors monitoring cellular ionic and bioenergetic balance in order to preserve cardiac homeostasis during metabolic stress situations [23, 54].

More recently, a great deal of interest has focused on K_{ATP} channels as underlying mechanism for exercise-induced cardioprotection. Convincing data exist demonstrating that short-term [26] and long-term exercise [25, 110] increase the expression of cardiac sarcolemmal K_{ATP}, and that pharmacological blockage of mitochondrial and sarcolemmal K_{ATP} channels impairs cardioprotection [25, 111–114].

Interestingly, sex specific levels of K_{ATP} channel expression were reported [115], suggesting varying degrees of exercise-induced cardioprotection between genders. Due to estrogen, female animals have higher levels of K_{ATP} channels, which subsequently provide enhanced cardioprotection against IRI following exercise [23].

Although association of exercise-induced cardioprotection and K_{ATP} channel opening is well established, the intracellular signaling cascade triggered by exercise and responsible for the opening of these channels remains unclear [54]. It is believed that phosphorylation of K_{ATP} channels by protein kinases, especially PKC via activation of opioid receptors, results in channel activity modulation [116, 117]. Previous data indicate that inhibiting both K_{ATP} channels and PKC do not provide additional protection, suggesting that activation of PKC and K_{ATP} channels are different components of the same signaling pathway [23].

5.4 Opioids System

Opioid receptors belong to the G-protein coupled receptor family and are traditionally known by their anti-nociceptive effect. However, more recently, it was discovered that an exogenous opioid ligand (morphine) apart from treating pain associated with myocardial infarct, may also help in reducing myocardial infarct size area. Since then, the putative negative inotropic action of opioid receptors has gained importance [116].

Interestingly, it is well established that stressful situations (e.g. ischemia and exercise) increase endogenous opioid peptide levels [11, 118–121]. Howlett et al. [122], for instance, observed that an acute bout of treadmill exercise markedly increased beta-endorphin (an opioid ligand) levels in young women either before and after an 8-week exercise training. In theory, this response could act as a compensatory mechanism aiming to counteract the high levels of catecholamines released during these stressful situation, and thus minimize potential damage in the heart.

Recent and exciting literature has provided evidence that opioid receptors may be another limb of exercise preconditioning [107]. Opioid receptor blockage precluded short [11] and long-term [80] exercise-induced ischemic tolerance. Posterior data [10, 77] have demonstrated that delta opioid receptor plays a key role in this response. Borges et al. [10] revealed that four consecutive days of exercise reduced the myocardial infarct size by approximately 34%. Exercise-induced cardioprotection against IRI was not blunted by pharmacological blockade of the kappa or mu

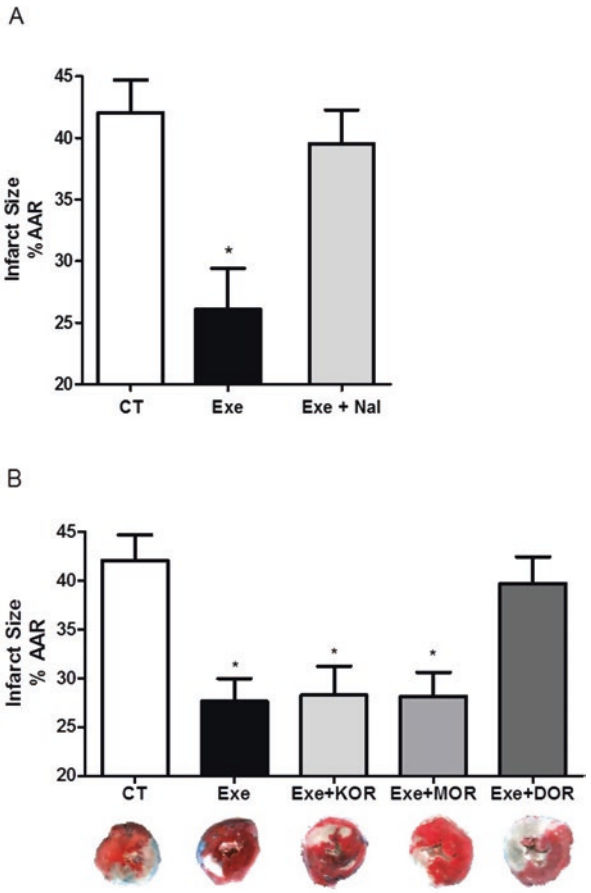

Fig. 10.5 Infarct size after ischemia reperfusion injury following non-selective (**a**) and selective (**b**) opioid receptor antagonists. CT (control group); Exe (exercised group); Exe + Nal (non-specific antagonist group); Exe + KOR (kappa opioid receptor antagonist group); Exe + MOR (mu opioid receptor antagonist group); and Exe + DOR (delta opioid receptor antagonist group). * $P < 0.05$ vs. CT (Source: DOI 10.1371/journal.pone.0113541.g002)

opioid receptors subtypes, whereas treating animals with nonselective (naloxone) and selective delta opioid receptor antagonist (naltrindole) abolished it (as seen in Fig. 10.5).

The protective effects of delta opioid receptor may be linked to the activation of PKC, which, in turn, opens sarcolemma/mitochondrial K_{ATP} channels [116], and triggers the cascade described in the previous section. Therefore, through a PKC-K_{ATP} channel pathway, delta opioid receptors would reduce mitochondrial electron chain damage, oxidative stress, MPTP opening and morphological changes in mitochondria [107].

6 Challenges

6.1 Animals Models

Notably, most studies available on exercise-induced cardioprotection are undertaken in experimental settings, where myocardium injury is caused in animals without established cardiovascular diseases. In other words, so far the cardioprotective effect of exercise against IRI has never been studied in animals presenting cardiovascular risk factors or diseases, such as hypertension, CAD or previous myocardial infarct. On the other hand, ischemic heart disease in humans is a complex disorder, which is usually caused by the combination of several cardiovascular risk factors and comorbidities (e.g. diabetes, heart failure, hypertension, altered coronary circulation and hyperlipidemia). These conditions together with the medications used against them lead to important changes in cellular signaling cascades that interfere in IRI and physiological responses to cardioprotective interventions, including exercise [123].

Importantly, it has been shown in animal models of myocardial infarction that various comorbidities, including hyperlipidemia and heart failure, can limit the efficacy of ischemic preconditioning and postconditioning [59, 123, 124]. Therefore, it is reasonable to assume that exercise-induced cardioprotection could also be affected in the presence of comorbidities. However, data in this sense are scarce. To the best of our knowledge, there is only one study that has focused on this issue [125]. In this study, rats with type 1 diabetes mellitus underwent global ischemia and reperfusion following resistance training or low-intensity aerobic training or high-intensity aerobic training. The authors concluded that exercise-related cardiovascular protection was dependent on the exercise modality, whereby high-intensity aerobic-exercised diabetic rats demonstrated the greatest myocardial recovery against IRI. Unfortunately, as the authors did not include a nondiabetic exercised group, conclusions regarding the changes in exercise-induced cardioprotection due to diabetes per se cannot be made.

6.2 Translating Exercise-Induced Cardioprotection to Clinical Practice

To achieve exercise-induced cardioprotection, exercise intervention is required before a cardiac insult, which obviously cannot be predicted in humans, making clinical research even more difficult. This explains why most data on this matter is based on experimental research with animals.

However, to establish associations and clinical implications, a bulk of evidence has demonstrated a phenomenon known as “warm-up” or exercise-induced ischemic preconditioning, in which exercise-induced ST-segment changes of patients

with CAD is markedly attenuated in the second of sequential maximal exercise sessions [126, 127]. An interesting finding was reported by Lambiase et al. [57], who submitted CAD patients to several maximal exercise tests prior to percutaneous coronary intervention and observed that the expected ST-segment elevation was attenuated.

Taken together, these data suggest that exercise preconditioning might be efficient in the promotion of clinical cardioprotection, reducing cardiac damage of patients under cardiovascular risk factors as well as those who suffered a cardiac event.

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Chapter 11
Experimental Evidences Supporting the Benefits of Exercise Training in Heart Failure

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Abstract Heart Failure (HF), a common end point for many cardiovascular diseases, is a syndrome with a very poor prognosis. Although clinical trials in HF have achieved important outcomes in reducing mortality, little is known about functional mechanisms conditioning health improvement in HF patients. In parallel with clinical studies, basic science has been providing important discoveries to understand the mechanisms underlying the pathophysiology of HF, as well as to identify potential targets for the treatment of this syndrome. In spite of being the end-point of cardiovascular derangements caused by different etiologies, autonomic dysfunction, sympathetic hyperactivity, oxidative stress, inflammation and hormonal activation are common factors involved in the progression of this syndrome.

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Together these causal factors create a closed link between three important organs: brain, heart and the skeletal muscle. In the past few years, we and other groups have studied the beneficial effects of aerobic exercise training as a safe therapy to avoid the progression of HF. As summarized in this chapter, exercise training, a non-pharmacological tool without side effects, corrects most of the HF-induced neurohormonal and local dysfunctions within the brain, heart and skeletal muscles. These adaptive responses reverse oxidative stress, reduce inflammation, ameliorate neurohormonal control and improve both cardiovascular and skeletal muscle function, thus increasing the quality of life and reducing patients' morbimortality.

Keywords Benefit • Exercise • Heart failure • Mortality • Outcomes

1 Introduction

Heart failure (HF) is a syndrome of poor prognosis in which patients present dyspnea and exercise intolerance due to the lack of the heart capacity of in maintaining the cardiac output required to preserve the metabolic needs of the organism. As a common end point for many cardiovascular diseases, more than 20 million people worldwide are estimated to have HF. This scenario tends to worse mainly because of the higher life expectancy and the increasing mean age of the population. The impairment of the cardiac function is the most classical mechanism described in this syndrome. Cardiac dysfunction can be of two types: a systolic and/or a diastolic dysfunction. Whilst most patients show both dysfunctions, there is usually a predominant pattern. The predominance of the systolic dysfunction, characterized by an inadequate emptying of the ventricle, defines a HF with reduced ejection fraction (HFREF). When the diastolic dysfunction (characterized by an inadequate relaxation and filling of the ventricle) predominates, it is called HF with preserved ejection fraction (HFPEF). Nowadays, our knowledge regarding HFREF is much wider when compared to that of HFPEF. HFPEF, however, is showing an increasing prevalence, being usually predominant in the elderly people and women. Around half of the HF patients experience HFPEF; unfortunately, none of the current therapies used to treat HFREF have shown good results in treating HFPEF patients. Besides the pharmacological therapies, aerobic exercise training has also been used to treat HF. Similar to other treatments, the current knowledge of the effects of exercise training in HF is predominantly focused in HFREF, which will be the focus of the present chapter.

This chapter will start with a brief overview on the pathophysiology of HF. Then, the effects of aerobic exercise training with focus on its benefits on neurohormonal control as well as its effects to improve cardiac and skeletal muscles functions will be discussed.

2 Overview on the Pathophysiology of the HF

The immediate response to a myocardial aggression, leading to decreased cardiac output, is the activation of compensatory neurohormonal mechanisms. Peripheral sensors, such as the baroreceptors and the cardiopulmonary receptors detect the alterations in arterial pressure, atrial distention and ventricular contractile function, which are integrated in central autonomic areas triggering the activation of several neurohormonal systems, the most important being the sympathetic nervous system, the renin-angiotensin-aldosterone system and the secretion of vasopressin [23]. In the early HF phase, these compensatory mechanisms aim to increase cardiac contractility and heart rate, in order to normalize the reduced cardiac output. However, their continuous activation induces an elevated peripheral resistance, with a consequent increase in the arterial blood pressure. Simultaneously an increased venous constriction and water/salt retention activated by the neurohormonal mechanisms, coupled with angiotensin II-induced increase in water intake, will result in a higher pre-load, activation of the Frank-Starling mechanism and increased ventricular contractility, which characterize the initial compensated phase of HF [23].

While the Frank-Starling mechanism is critically important in regulating cardiac output in normal conditions, in the presence of myocardial dysfunction its effects are greatly impaired. As the ventricle is incapable of ejecting proper volume during the systolic phase of the cardiac cycle, the heart will enter in the subsequent diastolic phase with increased residual blood volume, which, in addition to increased venous return, results in an even high pre-load. In the next cycle, again the heart is incapable of ejecting the proper systolic volume, leading the ventricle to work continuously under elevated filling pressures. In this condition, the heart works constantly in the right end of the Frank-Starling curve, showing minimal alterations in the cardiac output in response to increases in the pre-load. Additionally, the failing heart shows a decrease in the peak cardiac output of the Frank-Starling curve, further decreasing the relevance of this mechanism for the compensation of cardiac failure [147].

Along with the neurohormonal activation and the Frank-Starling mechanism, a third compensatory mechanism in HF is the ventricular hypertrophy. Left ventricle dilatation and/or sustained elevations in after-load result in higher wall stress. Both neurohormonal signaling and wall stress induce a hypertrophic response in cardiomyocytes and fibroblasts, thus leading to hypertrophy and extracellular matrix deposition. The pattern of this response depends on the type of stimulus applied to the ventricle: volume overload will result in eccentric hypertrophy with the maintenance of the wall thickness, while pressure overload results in concentric hypertrophy with increase in wall thickness [59]. While these adaptations at the beginning might help to reduce wall stress and maintain ventricular function, the exhaustion of this mechanism by the persistence of the injury triggers the chamber dilation and the reduction of its contractile function.

In spite of the importance of these mechanisms in the maintenance of the organism homeostasis in the acute HF, the persistence of such aggression, leading to a chronic activation of neurohormonal systems, will result in further deterioration of the cardiac function. The excessive activation of sympathetic, renin-angiotensin-aldosterone and vasopressin systems results in maladaptive responses of the myocardium, inducing apoptosis [79] and abnormal function even in the viable myocardium. Otherwise, the viable myocardium subjected to chronic neurohormonal stimulation shows impaired calcium handling and abnormal production and use of high-energy phosphates and reactive oxygen species [23, 41]. Sympathetic hyperactivation induces desensitization, thus reducing the capacity of the heart to respond adequately to autonomic stimuli. Catecholamines, angiotensin II, aldosterone and inflammatory cytokines altogether can trigger apoptotic responses in cardiomyocytes [79]. The worsening of cardiac function causes further stimulation of the neurohumoral systems, resulting in a deleterious positive feedback mechanism. This feedback loop of progressive worsening in cardiac function and compensatory increases of neurohumoral activation will eventually reach a limit when the cardiovascular system can no longer maintain an adequate perfusion of the organism, resulting in the HF syndrome.

3 Mechanisms Conditioning the Benefits of Exercise Training in HF-Neurohormonal Systems

3.1 Autonomic Nervous System

Autonomic nervous system dysfunction is a hallmark for HF. The exaggerated sympathetic activation simultaneously with withdrawal of vagal outflow drives the organism towards progressive worsening of cardiac function. Several methods and models of HF have been used to assess and confirm sympathetic nervous system (SNS) hyperactivity in animal models of HF: sympathetic nerve recordings [39, 135], dosage of plasma cathecolamines [123], norepinephrine turnover [122], immunohistochemistry in brain autonomic areas [69], as well as functional recordings [69]. The relevance of SNS in the pathophysiology of the HF is highlighted by the great impact of blocking sympathetic hyperactivity in reducing the mortality of HF patients [22, 53]. Exercise training, on the other hand, is capable of reducing or even normalizing SNS activity in HF animals [69, 185]. Even in patients that are already in the use of β-blockers, exercise training can induce further reductions in sympathetic nerve activity [48].

Many mechanisms have been proposed to explain the SNS dysfunction in HF. Impairment of inhibitory and hyperactivation of excitatory reflexes controlling the SNS outflow were pointed as important mechanisms leading to sympathetic hyperactivity in HF. Indeed, reduced sensitivity of the sympathoinhibitory arterial baroreflex [39] and cardiopulmonary reflexes [128] and increased sensitivity from exercise pressor reflex [164] and other sympathoexcitatory reflexes such as the

carotid body chemoreflex [145] and the cardiac afferent sympathetic reflex [163] were found in animal models of HF. Exercise training can attenuate several of these reflex dysfunctions. HF animals submitted to chronic exercise training show increased baroreflex sensitivity [94, 111] through a mechanism that seems to be dependent on the parasympathetic nervous system [95]. Exercise training also ameliorates cardiopulmonary reflexes [128], attenuates carotid body afferent activity and normalizes the chemoreflex through mechanisms dependent on NO and angiotensin signalling [91]. The exercise pressor reflex driven by metaboreceptors and mechanoreceptors afferents is also attenuated by exercise training, which prevents the sensitization of those receptors [164, 165].

Second order neurons in the nucleus tractus solitarii (NTS), the first synaptic relay of peripheral receptors in the central nervous system, receive barosensitive and chemosensitive inputs and project to brainstem areas controlling vagal (nucleus ambiguus, NA, and dorsal motor of the vagus, DMV) and sympathetic (caudal and rostral ventrolateral medulla, CVLM and RVLM, respectively) outflow to heart and vessels [36, 106]. Upon loading of baroreceptors, NTS is activated and increases the firing of NA and DMV pre-ganglionic parasympathetic neurons projecting to the heart; NTS also activates gabaergic inhibitory neurons within the CVLM that project and inhibit RVLM premotor neurons projecting to sympathetic pre- and postganglionar neurons innervating the heart and vessels [108]. As a consequence, venous return, cardiac output and peripheral resistance are reduced decreasing arterial pressure, which returns to control levels [107, 108]. When peripheral chemoreceptors are activated (reduced PO_2 and pH, increased PCO_2), the firing of NTS chemosensitive neurons directly excite the RVLM premotor neurons augmenting sympathetic outflow and increasing blood pressure [130, 171]. Opposed responses are observed to baroreceptors unload and during reduced activation of peripheral chemoreceptors, respectively. Brainstem integration of cardiovascular control is continuously modulated by preautonomic neurons located in the paraventricular nucleus of hypothalamus (PVN) and other supramedullary pathways [108, 149]. Considering the role of brainstem and supramedullary autonomic nuclei in the control of sympathetic and parasympathetic activity, it makes sense that plastic and functional changes in these nuclei could condition both deleterious and benefic autonomic adaptations to HF and training, respectively.

Studies in HF animals described significant reductions in the nitric oxide content (NO, a sympathoinhibitory molecule) within the NTS [67, 140], increased expression and higher functional response to AT1 receptors blockade [166]. Indeed, augmented availability of angiotensin II was proposed to be one of the mediators of sympatoexcitation in the brain. Indeed, angiotensin converting enzyme (ACE, responsible for the conversion of angiotensin I to angiotensin II) gene and protein expression is elevated and that of angiotensin converting enzyme 2 (ACE2, which metabolizes angiotensin II to angiotensin-(1–7)) is reduced in autonomic areas of the hypothalamus (PVN) and brainstem (NTS, RVLM) of chronic HF rabbits [73]. Coherently, exercise training, by reversing ACE/ACE2 ratio, is able to attenuate the increased angiotensinergic signaling in these nuclei [73]. Other experimental studies investigating the sympathetic hyperactivity in HF found increased angiotensinergic

[182] and glutamatergic [90] and decreased GABAergic [30] and NO [177] signaling within the PVN. Again exercise training reduced sympathetic overactivity simultaneously with decreased angiotensinergic [73, 182] and glutamatergic [77] and increased GABAergic [121] and NO [181] signaling in the PVN. Similar profile was observed within the RVLM, the main nucleus controlling the sympathetic outflow to the cardiovascular system: increased glutamatergic [167] and decreased NO signaling [67] simultaneously with an imbalance between AT1 and AT2 receptors [51], which contribute to sympatoexcitation in HF animals. All these alterations are attenuated by exercise training [73].

Apart of numerous studies confirming the role of sympathetic outflow in the genesis of cardiovascular deficits in HF, as well its withdrawal in the improvement of circulatory control in trained HF animals, the parasympathetic, the counter-regulatory axis of the autonomic nervous system whose activity is depressed in HF patients and animals [18, 69] has received much less attention. Although there is evidence that low vagal activity is a predictor of high mortality rates [34, 82], pharmacological activation of vagal outflow is not generally recommended given the several side effects of cholinergic drugs and the lack of drugs capable of specifically stimulating the vagal activity to the heart. So, the impact of the parasympathetic nervous system is not as clear as the effects of the sympathetic activity in HF. Pharmacological stimulation of parasympathetic tonus with pyridostigmine improves cardiac and circulatory parameters in HF rats [84, 137]. In chronic HF the increased vagal activity through parasympathetic nerve stimulation has shown to be effective to improve prognosis in animals [89, 179] and patients [37, 146]. However, in large randomized trials this intervention failed to show significant results [57].

Besides knowing that HF animals show alterations in parasympathetic ganglia and depressed parasympathetic activity [17], information regarding the mechanisms leading to vagal dysfunction in HF are lacking. In a recent paper we observed that decreased parasympathetic tonus in HF rats is positively correlated with the reduction of choline acetyl transferase (ChAT) positive neurons in the NA and DMV and that training-induced improvement of parasympathetic control of the heart is accompanied by a significant increase in the number and density of ChAT-positive neurons within these nuclei [69]. Figure 11.1 illustrates these findings showing in addition that elevated basal heart rate, which is driven by the increased sympathetic outflow to the heart in HF sedentary rats, is reduced and driven by the augmented parasympathetic tonus in trained HF rats. Our data also confirmed that increased sympathetic activity in HF sedentary rats is accompanied by augmented dopamine β-hydroxylase immunoreactivity (DBH*ir*) within the RVLM and that exercise training reduces both [69]. However, the correlation between sympathetic tonus and DBH*ir* within the RVLM does not attain significance [69]. These observations reinforce the potentiality of training to improve vagal control of the heart in HF individuals, with the advantage to avoid noxious side effects that accompanied pharmacological therapies. In spite of our still limited knowledge regarding the parasympathetic axis of autonomic nervous system in the treatment of chronic HF, exercise training seems to be an essential therapeutic tool to normalize vagal dysfunction in this syndrome.

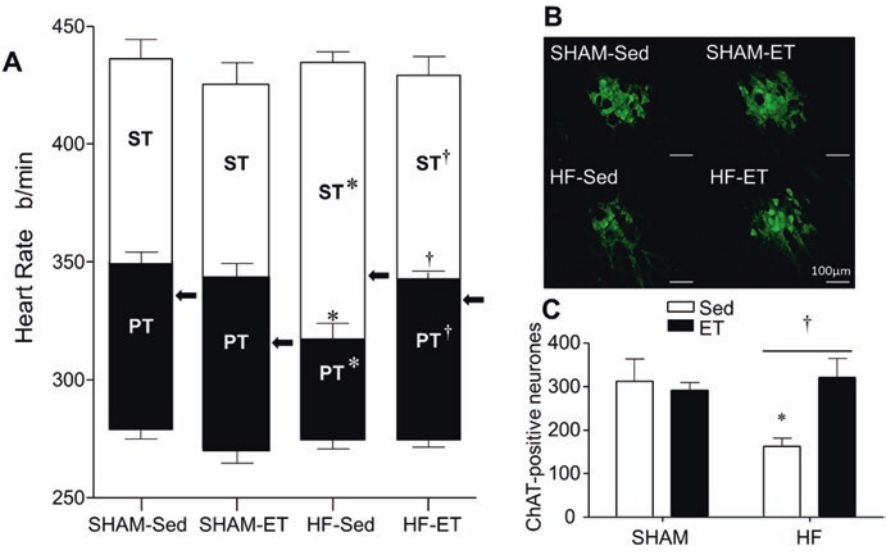

Fig. 11.1 (**a**) Comparison of cardiac sympathetic (ST, open bars) and parasympathetic tonus (PT, filled bars), intrinsic heart rate (intersection between ST and PT) and resting heart rate (indicated by arrows) in infarcted (HF) and SHAM rats submitted to sedentary (Sed) and training (ET) protocols. Significances ($P < 0.05$) * vs. SHAM; † vs. Sed. (**b**) Photomicrographs comparing the effects of heart failure and exercise training on Choline Acetyl Transferase (ChAT) immunoreactivity within the nucleus ambiguus pars sub-compacta of SHAM and HF rats submitted to sedentary (Sed) or training (ET) protocols. (**c**) Number of ChAT-positive neurones in pars sub-compacta of the nucleus ambiguus. Significant difference ($P < 0.05$): * vs. SHAM; † vs. respective Sed controls (Modified with permission from Ref. [69]

Therefore, by attenuating sympathoexcitation and by restoring the vagal control of the heart, exercise training is able to restore autonomic balance in HF individuals, even in the persistence of ventricular deficits, therefore improving its prognosis besides reducing mortality rates.

3.2 Renin-Angiotensin-Aldosterone System (RAAS)

Along with the autonomic nervous system, the RAAS is an essential key in the understanding of HF pathophysiology. The RAAS is a complex system composed of several regulatory and counter-regulatory molecules that act in order to control the water and salt balance and the arterial blood pressure. Viewed in the past as a hormonal circulating system, it is now accepted as an important local regulatory system present in all tissues, able to control specific tissue functions independently of the circulating RAAS. This hormonal/local system exerts its functions through 2 axes: the ACE-angiotensin II-AT1 receptor axis with vasoconstrictor, proliferative and pro-inflammatory effects and the ACE2-angiotensis-(1–7)-Mas receptor axis, with opposite vasodilator, anti-proliferative and anti-inflammatory effects. In

addition to increased angiotensin II availability within the brain leading to increased sympathetic outflow [94, 111], ACE-angiotensin II-AT1 receptor axis is hyperactivated in HF [13, 58, 73, 94, 125], the increased angiotensin II levels being responsible for fibroblasts' proliferation and myocardium hypertrophy, thus facilitating the worsening of cardiac function in an already dysfunctional heart [139].

The efficacy of RAAS blockade (renin and ACE inhibitors, AT1 receptors' antagonists, aldosterone receptors' antagonists) in reducing the neurohormonal activation of the heart and reducing mortality [83] highlight the importance of these therapeutic tools to improve prognosis in HF patients. Importantly, exercise training is effective in attenuating RAAS activity not only in the brain, but also in peripheral tissues, thus avoiding additive deleterious effects in the progression of HF. Indeed, HF animals submitted to exercise training show decreased plasma angiotensin II concentration [94] simultaneously with reduced tissue content in the heart [125], skeletal muscle [58] and brain [51, 73, 182]. Despite accumulating evidence for the importance of RAAS in HF and the benefits of exercise training in reducing its activation in several peripheral tissues, the most abundant information available was obtained in the central nervous system. Exercise training, by modulating RAAS activity can correct/normalize blunted reflexes that regulate autonomic circulatory control, such as the baroreflex [111] and the carotid body chemoreflex [91]. In addition, as described before, the enhanced angiotensinergic signaling in autonomic areas of HF individuals (increased AT1 receptors and ACE expression, decreased ACE2 expression, etc.) [61, 73, 182] determining sympathoexcitation is corrected by exercise training.

Angiotensin II-induced increases in sympathetic activity are mediated, at least in part, by increases in oxidative stress [49, 183] and exercise training has been shown to decrease sympathetic hyperactivity by reducing oxidative stress: it increases the expression of antioxidant enzymes in the brain and other tissues [50, 85, 93, 154], thus attenuating intracellular signaling triggered by angiotensin II.

Aldosterone, a mineralocorticoid secreted in response to angiotensin II signaling that is mostly known for its role in sodium reabsorption in the kidney. Nonetheless, aldosterone receptors are present in the heart [96, 124], as well as in vessels [96, 104] and brain [176]. In the heart of HF individuals, aldosterone induces marked cardiac fibrosis worsening the cardiac function [24, 133]. On the other hand, blockade of aldosterone effects by mineralocorticoid receptors antagonists has been shown to reduce mortality of HF patients [103]. There is scarce information regarding the effects of exercise training on aldosterone effects in HF. Braith et al. [21] and Wan et al. [162] have shown that exercise training reduces circulating levels of aldosterone, thus contributing to attenuate its deleterious effects in HF.

3.3 Inflammatory Response

The increased inflammatory profile also plays an important role in the pathophysiology of the HF. Plasma levels of pro-inflammatory cytokynes, such as tumor necrosis factor - alpha (TNF-α) and interleukins (IL) as IL-1β, IL-6 and IL-18, are elevated

in several tissues of HF individuals while anti-inflammatory cytokines, such as the IL-10, are reduced [60]. Intact rats chronically infused with TNF-α showed depressed cardiac function and left ventricle dilatation, a pattern that resembles the effects induced by HF [20]. These effects are partially reversed by stopping the TNFα infusion [20]. A murine model that overexpresses TNF-α in the heart also develops cardiac hypertrophy and dilatation, with reduced ejection fraction and pulmonary congestion, a phenotype very similar to HF [81]. Elevated levels of TNF-α is also related to the skeletal muscle apoptosis found in HF rats [35]. Dysfunction of human cardiomyocytes submitted to ischemia-reperfusion injury is attenuated by simultaneous inhibition of IL-1β and IL-18 [129]. In rats the chronic exposure to IL-6 induces myocardial fibrosis, cardiac concentric hypertrophy and diastolic dysfunction [102] while IL-6 knockout mice submitted to pressure overload show attenuation of both left ventricle hypertrophy and cardiac dysfunction [180].

While the relevance of the immune response in the context of HF is clear, studies aiming to modulate it with drugs administration are still ensuing. A trial using anti-TNF-α antibodies showed no improvement and had to be stopped because of increased mortality in the group receiving the higher doses of the drug [32]. As reviewed by Gullestad et al. [60], other studies using different approaches to modulate the immune response in HF showed that with few exceptions those treatments are neutral or even harmful, calling our attention for the need to expand the knowledge in this field. In contrast exercise training has shown significant effects in reducing pro-inflammatory profile in HF in rats and patients. HF rats submitted to exercise training show increases in plasma levels of the anti-inflammatory cytokine IL-10 [119] and reduction of LPS-stimulated TNFα production by macrophages [15]. Exercised HF patients show reduced plasma levels of TNF-α and its receptors (sTNF-RI and sTNF-RII), IL-6 and its receptor (sIL-6R) and of the apoptosis inducer sFasL [3]. Markers of the monocyte/macrophage system granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage chemoattractant protein-1 (MCP-1) are also reduced [2]. These findings indicate that exercise training is a better choice than recombinant antibodies and/or pharmacological blockade to modulate immune response in HF.

4 Mechanisms Conditioning the Benefits of Exercise Training in HF – Cardiovascular System

4.1 Heart

As commented above, impairment of cardiac function is a hallmark of HF. The progression of the syndrome induces progressive deleterious remodeling of the heart leading to dilation of the chambers and loss of its elliptical shape [78]. Exercise training is able to prevent most of these alterations. Some studies have shown amelioration of cardiac function or reverse remodeling in trained HF animals [77, 182] and patients [42, 64, 157]. Others have found no significant effects in both animals

and patients [61, 73, 94, 136, 143]. These discrepancies could result from differences in the intensity, duration and type of the exercise protocol used [169]. Therefore, the beneficial effects of exercise training on myocardial remodeling and function seems to be only mild. Nonetheless, exercise training is capable improve other deficits induced by HF.

The impaired coronary blood flow and coronary reserve in HF are improved by exercise training, which activates myocardial angiogenesis [87, 143]. This finding is of relevance since the high coronary flow reserve has significant prognostic value in the context of HF [132]. Decreased coronary blood flow in HF is related to an increased production of reactive oxygen species in the coronary arteries and decreased levels of antioxidant enzymes [31], leading to increased NO scavenging and impaired endothelial NO synthase (NOS) function [16, 168]. Excessive oxidative stress, as demonstrated by increased levels of reactive oxygen species and decreased levels of antioxidant enzymes, also affects the myocardium itself [65, 66, 70]. The consequences of this dysfunction is the injury of cardiomyocytes, with contractile abnormalities [72], impairment of the proteasome, leading to accumulation of misfolded proteins [46], and eventually culminating in cell death. Exercise training induces cardioprotection through the reduction in oxidative stress simultaneously with the increase of antioxidant enzymes [12], thus restoring the cellular protein quality control [29].

Another feature of HF is impaired Ca^{2+} handling. The calcium homeostasis within cardiomyocytes is regulated by several proteins. Special attention has been given to those responsible for the control of the Ca^{2+} uptake and release within the sarcoplasm and sarcolemma. Those include the sarcoplasmic reticulum Ca^{2+}ATPase (SERCA2) and its regulator phospholamban (PLN), the ryanodine receptor, Ca^{2+} channels, and the Na^{+}/Ca^{2+} exchanger. While it is consensual that HF leads to Ca^{2+} handling dysfunction and excitation-contraction uncoupling, the mechanisms leading to those alterations are very complex and studies show conflicting results [11, 98]. Nonetheless, it seems that exercise training is able to ameliorate the HF-induced Ca^{2+} handling alterations, whichever directions they occur [76, 101, 134, 152, 170].

The heart in HF, submitted to excessive sympathetic signaling, show β-adrenergic receptor desensitization [56]. This results from a reduction in the density of β_1-adrenergic receptor, a decreased β_1 / β_2 ratio [26] and uncoupling of β_1-adrenergic receptor from the G_s protein caused by enhanced βARK expression [156]. Exercise training can attenuate this desensitization thus increasing β-adrenergic response [87], likely through increases in the expression of β_1-adrenergic receptors and cAMP levels [38, 87]. Therefore, exercise training can restore cardiac contractility reserve in HF.

HF also results in a dysfunction of the sinus node pacemaker cells leading to decreased intrinsic pacemaker heart rate (see Fig. 11.1) [69, 141, 174]. This sinus node dysfunction is characterized by increased recovery time and intrinsic cycle length, a caudal shift of the pacemaker location and slower sinoatrial conduction [141]. Molecular alterations that might explain these alterations include widespread changes in the expression of ion channels, gap junction channels, Ca^{2+}, Na^{+}, and H^{+}-handling proteins and receptors [174]. This sinus node dysfunction, along with

the β-adrenergic desensitization, lead the organism to require a higher sympathetic activation to maintain a similar heart rate when compared to normal subjects [69]. Exercise training also reverses this dysfunction, restoring intrinsic pacemaker heart rate of HF rats to similar levels when compared to control animals [69]. Whether the other mechanisms (for instance the anatomical change in the pacemaker location) are also corrected it remains to be investigated.

4.2 Endothelium

Impaired endothelium-derived vasodilatation is characteristic of HF [80]. This dysfunction is caused by reduced production of endothelial-derived relaxing factors, most notably NO [74, 131] and increased levels of endothelin [88]. Increased production of both reactive oxygen species (that inactivates NO) [16] and pro-inflammatory cytokines (such as the TNF-α that decreases endothelial NOS activity) [4, 172] are among the mechanisms that lead to the depletion of NO. The relevance of endothelial dysfunction in HF is of great importance and its severity can predict deleterious outcomes [105]. Exercise training increases NOS expression, restores NO production and decreases oxidative stress [75, 158] improving endothelium-mediated dilation and attenuating deleterious alterations. Exercise can also restore the number and function of endothelial progenitor cells [142, 144] and increase the levels of proangiogenic cytokines, such as the vascular endothelial growth factor (VEGF) and the stromal cell-derived factor (SDF-1) [144], suggesting that exercise also ameliorates angiogenesis.

5 Mechanisms Conditioning the Benefits of Exercise Training in HF – Skeletal Muscle

5.1 Skeletal Myopathy

The HF-related skeletal myopathy can induce a severe syndrome known as cardiac cachexia. This syndrome is defined by an ongoing loss of skeletal muscle mass that cannot be fully reversed by conventional nutritional support and that leads to progressive muscle functional impairment. This severe clinical complication is also observed in many other chronic disease conditions, such as cancer, diabetes and HIV infection, affecting different types of skeletal muscles that are involved not only in force production, but also in posture maintenance and respiration. Epidemiological data demonstrate that in comparison with non-cachectic patients, the average stay at the hospital for cachectic patients is twice longer, and cost 70% more [7]. Thus, the reduced muscle mass and muscle dysfunction in HF are strongly associated with a reduced quality of life and a poor prognosis. Curiously, no specific

therapy are current available to block or attenuate the process of HF-related skeletal myopathy, leading the patients to develop cardiac cachexia.

In addition to muscle mass loss and decreased muscle function, HF-related skeletal myopathy has been characterized by capillary rarefaction, mitochondrial dysfunction, altered myofiber phenotype (causing a shift from type I slow twitch toward type II fast twitch myofibers) and reduced muscle endurance [160]. Together, these features contribute to the increased fatigability leading patients to dyspnea, fatigue and exercise intolerance.

The sustained hyperactivities of SNS and RAAS, described in the previous topics are directly associated with the pathogenesis of HF, can directly contribute to the changes in morphofunctional features related to skeletal myopathy. One of the main pharmacological therapies of HF is the blockade of the sympathetic and RAAS hyperactivity, through the use of β-blockers and ACE inhibitors or AT1 receptor antagonists, respectively; however, the effect of these treatments on skeletal myopathy has not been clarified yet. In contrast, it was already demonstrated that aerobic exercise training (AET) emerges as a potent non-pharmacological strategy to counteract HF-related skeletal myopathy and the evidences from basic science are strong enough to recommend it as an adjuvant therapy.

5.2 Sympathetic Hyperactivity and Skeletal Myopathy

The sympathetic activation in skeletal muscle tissue is mediated by β-adrenergic receptors (β-AR) and this activation can improve muscle regeneration process [151], increase force production, promote a shift toward type II glycolytic myofibers and increase muscle mass [99]. This hypertrophic response was described by studies which used β-AR agonists, such as *clenbuterol* and *formoterol* (selective β_2-AR agonists) and *isoproterenol* (a nonselective β-AR agonist) [71, 99, 173]. The cellular mechanisms involved in this process include, an inhibition in muscle proteolysis, mainly by ubiquitin-proteasome system (UPS), concomitantly with an increased protein synthesis, mainly associated with Insulin Like Growth Factor1/Phosphoinositide-3-kinase/Akt-protein kinase B/mammalian-mechanistic Target Of Rapamycin (IGF-1/PI3K/Akt/mTOR) signaling pathway [114–116].

Based on aforementioned hypertrophic effect, β-AR activators were prescribed to counteract the HF-related muscle myopathy in late 80's decade. In fact, some beneficial effects of β-AR agonists on muscle mass were observed; however, tachycardia was reported as a side effect [110]. Tachycardia occurred due to the β_1-AR related cardiac effect, while the hypertrophic effect of β-AR activators was demonstrated to be specific to selective β_2-AR agonists which would be more efficient to combat skeletal myopathy [52]. In this sense, our group observed that β_2-AR knockout mice displayed exercise intolerance and a severe muscle atrophy after myocardial infarction induced-HF [161]. One possible explanation is that in previous stages of HF, increased sympathetic activity through the activation of β_2-AR could be able to delay the onset of muscle proteolysis.

This seems to be the case in a mice model of sympathetic hyperactivity induced-HF, which was largely used in many studies of our group. At 3 months of age, although no signs of HF were present, these animals displayed sympathetic hyperactivity associated with *plantaris* muscle hypertrophy mediated by β_2-AR activation [10]. In the same mouse model, when HF syndrome turned severe, the *plantaris* atrophy and skeletal myopathy became evident. Therefore, while activation of β_2-AR by β_2-agonists seems to counteract skeletal myopathy in early stages of the syndrome, long-term and sustained activation of SNS leads to HF-related skeletal myopathy, which might be related to β_2-AR desensitization and downregulation reducing its anabolic effects. In fact, sympathetic hyperactivity besides being one hallmark of HF, it also contributes to the development of the skeletal myopathy [136].

5.3 Renin-Angiotensin-Aldosterone System Hyperactivity and Skeletal Myopathy

Angiotensin II (Ang II) is the main effector molecule of the system and its high levels are also a hallmark of HF leading to vasoconstriction, pro-inflammatory effects and reduced muscle regenerative capacity [45, 175]. High levels of Ang II induce protein breakdown and decrease the levels of skeletal muscle protein synthesis, leading to cardiac cachexia [47]. In addition to its direct effects on skeletal muscle, the indirect effects of Ang II can also contribute to muscle atrophy, due to its role in regulating circulating hormones and inflammatory cytokines. In this sense, Ang II increases interleukine-6 (IL-6) cytokine levels leading to an imbalance in the ratio between skeletal muscle protein synthesis and protein degradation by inhibiting IGF-I/Akt/mTOR signaling pathway while activating UPS [178]. It was observed that Ang II, when infused in rodents through osmotic pumps for up to 2 weeks, significantly decreased systemic IGF-I levels. In addition, the animals presented reduction in body weight and daily food intake, which are directly related to cardiac cachexia [25].

In addition to ACE inhibitors or AT1 receptor blockers, vasodilator agents are commonly used as hypertensive therapy in HF syndrome. However, it was shown that only the compounds that act directly in RAAS are able to block the changes in circulating IGF-I and body weight reduction, indicating that Ang II induces cardiac cachexia through a pressor-independent mechanism [5, 25].

Thus, pharmacological inhibition of RAAS can be recommended to avoid exercise intolerance and increasing the quality of life related to an attenuated skeletal muscle myopathy. In fact, HF treatment with ACE inhibitors increases respiratory muscle strength in humans [33] and partially prevents HF-induced muscle myopathy in rodents [184]. The same features were observed for AT1 receptor blockers which, at least in part, can attenuate the reduced muscle force in HF syndrome [44].

Even though the therapy with inhibitors of RAAS has demonstrated some positive outcomes in HF-related skeletal myopathy, AET also emerges as a potential non-pharmacological adjuvant therapy modulating RAAS.

5.4 *Aerobic Exercise Training: An Important Non-pharmacological Treatment for HF-Induced Skeletal Myopathy*

The aerobic exercise training (AET) have been studied in its basis for more than 50 years and nowadays it is recognized as an efficient and safe strategy in order to prevent and/or treat several cardiovascular diseases [43]. The beneficial effects of AET in HF have been demonstrated in heart, neurohumoral systems and skeletal muscle tissue. Therefore, both European [40] and American [68] guidelines have agreed upon the recommendation of AET in combination with an adequate pharmacological treatment. Interestingly, the responsiveness of skeletal muscle to AET is higher than to pharmacological therapy, which highlights the importance of the AET as strategy to counteract HF-related muscle myopathy. As will be described below, data from basic science provide strong evidence for AET as a prominent strategy to prevent and/or revert muscle metabolic and contractile dysfunction in HF.

5.5 *Effects of AET in the Metabolism and Function of the Skeletal Muscle*

HF causes many metabolic changes in the skeletal muscle tissue [100, 127]. Those changes, such as a switch toward type II glycolytic myofibers and decreased mitochondrial density and function, trigger a reduced aerobic capacity leading to muscle fatigue and exercise intolerance. Indeed, a decrease in protein expression of PGC-1α (peroxisome proliferator-activated receptor gamma), a potent regulator of mitochondrial biogenesis, was observed in animal models of HF [159]. In contrast, AET is able to modulate those metabolic changes due to its capacity to improve the production and the utilization of energy substrates by the muscle cells in a more efficient way. Such improvements in muscle substrate supply and uptake are optimized by the enhanced blood supply to skeletal muscle tissue, once AET prevents HF-induced capillary rarefaction [62]. In addition, AET promotes a shift toward oxidative type I myofibers in skeletal muscle tissue, which improves its oxidative features [10].

Due to the HF-related cachexia, the skeletal muscle contractile function is also impaired in HF and these features are strongly associated with changes in Ca^{2+} handling. In fact, rodents with HF displayed low levels of sarcoplasmic Ca^{2+} associated with reduced rate of sarcoplasmic reticulum Ca^{2+} release and reuptake [97, 126]. These findings are also observed in patients, since a reduced Ca^{2+} release and reuptake associated with decreased dihydropyridine receptors and sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA)2a protein expression in *vastus lateralis* was observed [109].

Herein, AET shows its effectiveness by improving skeletal muscle Ca^{2+} handling. In fact, our group have demonstrated that AET at moderate intensity can improve

the net balance of Ca^{2+} handling proteins in *soleus* and *plantaris* muscle from sympathetic hyperactivity induced-HF mice, culminating in a better muscle function [28]. Interestingly, Ca^{2+} handling is also observed in HF patients since leg extension training was able to reduce Ca^{2+} leaking through ryanodine receptors in *vastus lateralis* muscle [112].

5.6 *Effects of AET in Neurohumoral Hyperactivity and for the Control of Skeletal Muscle Mass*

As previously mentioned, cardiac cachexia is considered an independent predictor of morbidity and mortality in HF patients and animal models. This syndrome is triggered by neurohumoral hyperactivity in association with impaired muscle function. Besides no specific therapy is available until now for treating muscle wasting in HF syndrome, AET can counteract the muscle myopathy by improving muscle function and metabolism (direct effect) or by attenuating neurohumoral hyperactivity (indirect effect).

Regarding neurohumoral hyperactivity, it was demonstrated that a 4-month period of moderate intensity AET leads to a significant reduction in muscle sympathetic nerve activity in HF patients [136]. Although the mechanisms behind this reduction are a topic under current investigation, some potential candidates were identified, such as afferent autonomic control coordinated by arterial baroreceptors, cardiopulmonary receptors and chemoreceptors [27, 150]. In fact, it was observed that AET is able to improve metaboreflex and mechanoreflex [6]. In addition, reduced AT1 receptors and normalized ACE levels in the brain of HF rodent models have been proposed as one of the possible mechanisms of reducing sympathetic hyperactivity by AET [186]. Indeed, it was demonstrated that AET reduces serum Ang II levels, and such effect is related to a lower sympathetic activity in HF [58, 117].

The neurohumoral hyperactivity is also associated with high concentrations of pro-inflammatory cytokines and muscle *redox* imbalance, which are involved in muscle catabolism. In fact, increased circulating TNF-α levels (a pro-inflamatory cytokine) were observed in patients with atrophy and muscle weakness [118]. Moreover, the increased muscle TNF-α expression contributes to the local protein degradation. The effects of TNF-α on HF-related skeletal muscle myopathy are mediated through the activation of a family of transcription factors known as nuclear factor kappa B (NF-kB), which regulate UPS [1]. Interestingly, AET is able to reduce serum TNF-α levels and plasma inflammatory markers in HF patients [2]. This response is accompanied by a reduced atrophy and improved muscle function. In addition, AET also reduces muscle expression of pro-inflammatory cytokines in HF patients [54].

The high levels of TNF-α in HF triggers an increase in reactive oxygen species (ROS) production which will ultimately lead to protein degradation by the UPS [92]. UPS is up regulated in HF due to its action in degradation of damaged proteins

in skeletal muscle [8]. The key effectors of the UPS are the enzymes known as E3-ligases (ubiquitin ligases), which couples activated ubiquitin to lysine residues on protein substrates conferring specificity to the system [92]. Two of these E3-ligases (Atrogin-1 and MuRF1) were already well described and their transcriptional activities are elevated in skeletal muscle tissue under various atrophic conditions; therefore, making them good markers of atrophy being known as *atrogenes* [86]. In fact, it was observed that AET reduces Atrogin-1 mRNA levels and normalizes proteasome activity in skeletal muscle from both rodent models and HF patients, highlighting the importance of AET to prevent UPS hyperactivity in HF [55].

On the other hand, protein synthesis is essential to the positive control of the skeletal muscle mass. Since IGF-I muscle levels are reduced in HF [63], the activation of IGF-I/Akt/mTOR signaling pathway could be considered a good strategy to counteract HF-induced muscle atrophy. In fact, it was demonstrated that muscle-specific IGF-I transgenic expression or gene transfer procedure in muscles can sustain muscle hypertrophy [113] and prevent muscle mass loss in different animal models of muscle atrophy, such as Duchenne dystrophy [14], dexamethasone injection [138], immobilization [155], Ang II infusion [153], and HF [148]. In this same line, it is known that Akt gene transfer procedure in skeletal muscles from rodents can induce hypertrophy and improve the regenerative process [120]. In addition, transgenic mice with muscle-specific overexpression of Akt displayed around 40% of increase in skeletal muscle mass accompanied by an improvement in force development [19]. Therefore, another possible strategy to increase the expression of elements from IGF-I/Akt/mTOR could be through AET, since it is able to revert the reduced muscle IGF-I expression in HF patients [148].

These results highlight the fact that AET re-establishes the skeletal muscle homeostasis attenuating atrophy, and this was recently demonstrated by our group using a mice model of sympathetic hyperactivity induced-HF. In order to verify whether AET could ameliorate the HF-related skeletal muscle myopathy, mice underwent to moderate intensity AET (5 days a week for 8 weeks) were evaluated. As expected, HF mice displayed atrophic *soleus* muscle in both type I and type IIa myofibers. Interestingly, AET was effective in attenuating this atrophy. This protective effect against muscle atrophy was associated with a reversion in exercise intolerance and an increase in motor performance. In addition, it was suggested, at least in part, that one of the possible mechanisms related with that improvement in skeletal muscle mass and function was the reestablished level of some components of IGF-I/Akt/mTOR signaling pathway [9]. However, up to now, no study investigated the real role of Akt, mTOR and any other downstream related proteins of that signaling pathway in skeletal muscle tissue during the development of HF.

Collectively, it has been demonstrated that AET is able to promote remarkable beneficial adaptations in skeletal muscle tissue during the development of HF syndrome. Therefore, it can be considered the hypothesis that AET is a powerful non-pharmacological therapy in order to prevent the onset of the HF-related skeletal myopathy and to avoid cardiac cachexia.

6 Conclusions

HF syndrome in different experimental models is accompanied by autonomic dysfunction, neurohormonal hyperactivity, oxidative stress and inflammation that trigger progressive worsening of the cardiac function and a severe skeletal myopathy, that leads to the loss of functional capacity and poor quality of life. These chronic deleterious HF-induced alterations are responsible for the high mortality rates exhibited by HF patients. Experimental studies have provided ample evidence regarding the benefits of aerobic exercise training in this pathology, as summarized in Fig. 11.2. Exercise training is highly efficient in ameliorating HF-induced dysfunctions by acting in the same pathways targeted by current standard pharmacological care (i.e. β-blockers, ACE inhibitors and angiotensin receptor blockers, aldosterone-receptor antagonists). In addition, exercise training has been shown to correct vagal outflow, inflammatory response and skeletal myopathy, improvements not yet obtained through available pharmacological therapy. These findings support the efficacy of aerobic exercise training in the treatment of chronic HF with of the advantage of avoiding side effects.

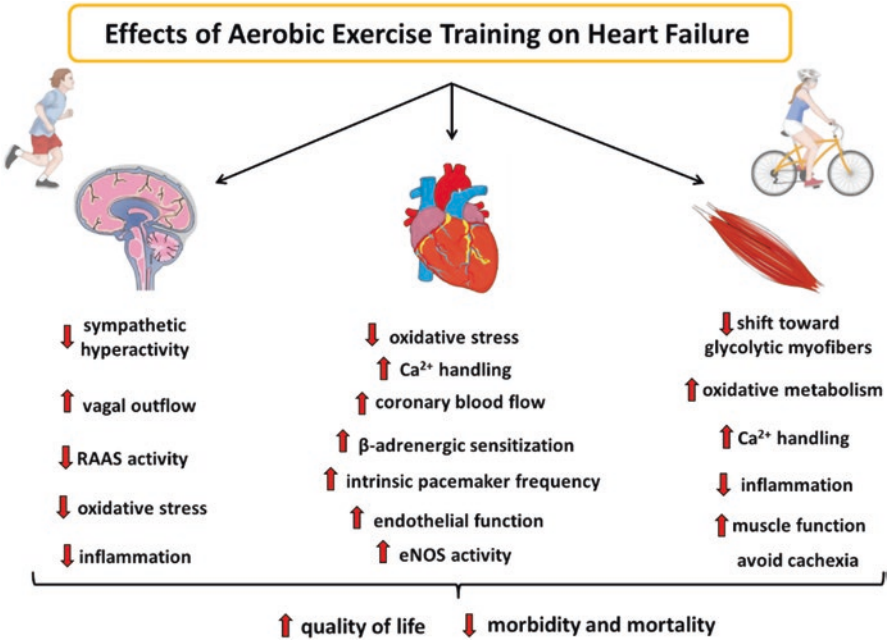

Fig. 11.2 The effects of aerobic exercise training on heart failure patients. eNOS, endothelial nitric oxide synthase, RAAS, renin-angiotensin-aldosterone system

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Chapter 12
Exercise Amaliorates Metabolic Disturbances and Oxidative Stress in Diabetic Cardiomyopathy: Possible Underlying Mechanisms

Ayman M. Mahmoud

Abstract Cardiomyopathy is a serious complication of diabetes mellitus and occurs independently of coronary artery disease or hypertension. It manifests as systolic/diastolic dysfunction and hypertrophy of the left ventricle and can lead to heart failure. Hyperglycemia can trigger a series of maladaptive stimuli and result in cardiac hypertrophy, fibrosis and reduced performance and contractility. The pathogenesis of diabetic cardiomyopathy is a multifactorial process that includes metabolic derangements such as increased oxidative stress, and altered non-oxidative glucose pathways and lipid metabolism. Exercise is a useful non-pharmacological strategy effective in the reduction of diabetes and obesity risk factors, and improvement of antioxidant defenses, mitochondrial function and physiological cardiac growth. It can amend multiple metabolic derangements and alterations in the diabetic heart. Therefore, figuring out the underlying mechanisms of exercise-induced beneficial effects could help to develop new treatment strategies for diabetic cardiomyopathy.

Keywords Oxidative stress • Cardiomyopathy • AGEs • Lipids

1 Introduction

Diabetes mellitus (DM) is one of the well-known risk factors for cardiovascular disease (CVD) and heart failure [1], and CVD has been reported to be the leading cause of morbidity and mortality in diabetic patients [2]. Forty-five years ago,

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Rubler et al. [3] have first described cardiomyopathy in a small cohort of diabetics with post-mortem adverse myocardial structural changes in the absence of hypertension, valvular complications or coronary arterial disease. Several experimental and clinical evidence suggested high predisposition of the diabetic subjects to cardiomyopathy [2]. Early impairments in diastolic function, and cardiomyocyte hypertrophy, apoptosis and fibrosis represent the characteristic features of diabetic cardiomyopathy (DCM) [2]. DCM manifests as systolic/diastolic dysfunction and hypertrophy of the left ventricle, therefore, increases the chances of heart failure [4, 5]. In addition, the higher occurrence of biventricular dysfunction in diabetic patients suggests that DM is an independent factor for cardiomyopathy [6, 7].

Exercise represents a useful non-pharmacological strategy for the prevention of type 2 DM and obesity [8, 9], and therefore of cardiovascular diseases. It is well established that exercise induces cardioprotective effect in the normal heart through several molecular mechanisms [10]. Multiple studies have demonstrated the beneficial effects of appropriate volume and intensity of exercise on cardiac dysfunction through the amelioration of left ventricle (LV) diastolic and systolic volumes, LV ejection fraction, ventilatory threshold, cardiac output and maximum oxygen consumption (VO_2max) [11–17]. The beneficial effects of exercise are not only linked to the reduced risk factors, but also associated with improved mitochondrial viability and antioxidant defenses, and activated physiological cardiac growth through cellular mechanisms other than those of pathological hypertrophy [18, 19]. This chapter highlights the metabolic derangements in the diabetic heart and how exercise training may influence the progression of diabetic cardiomyopathy, focusing on myocardial metabolism, and hyperglycemia-induced pathways and oxidative stress.

2 Exercise Improves Myocardial Metabolism

Metabolic flexibility and the ability to ensure adequate adenosine triphosphate (ATP) production rate are important features of the normal heart [20]. Lack of this flexibility contributes to the development of DCM, but the exact mechanism remains unclear [20]. Oxidation of fatty acids (FA) is the primary energy source for diabetic heart, despite the presence of hyperglycemia [21, 22]. In diabetes and obesity, myocardial FA uptake and oxidation increase while glucose oxidation decreases. Increased FA oxidation occurs through the activation of peroxisome proliferator activated receptor-α (PPAR-α) and induction of enzymes involved in transport and β-oxidation of FA [21–23]. Genetically modified mice mimicking the diabetic metabolic phenotype have been demonstrated to develop cardiac dysfunction [24, 25]. In addition, experimental and clinical studies have shown that altered cardiac substrate metabolism precedes ventricular dysfunction [22, 23]. Moreover, maintenance of FA oxidation has been reported to have beneficial effects in the diabetic heart [26–28]. Therefore, targeting myocardial metabolism may represent a therapeutic intervention for attenuating DCM [29].

Several studies have demonstrated the effect of exercise on substrate utilization in normal, obese and diabetic myocardium. In diet-induced obese rats, exercise increased insulin-stimulated phosphorylation of insulin receptor (IR), protein kinase B (PKB/Akt) and insulin receptor substrates (IRS-1 & -2) [30, 31]. However, the effect of exercise on myocardial substrate utilization has not been investigated in these studies [20]. Streptozotocin (STZ)-induced type 1 diabetic rats exposed to exercise training showed unaltered oxidation rate of FAs in the myocardium [32], despite the increased rates of glucose oxidation and glycolysis [12]. In agreement with these findings, Hafstad et al. [33] reported increased myocardial glucose oxidation with no changes in the rate of FAs oxidation after treadmill running in diet-induced insulin-resistant mice. On the contrary, treadmill running didn't affect glucose oxidation and glycolysis in the normal myocardium [12]. Another study conducted by Burelle et al. [34] demonstrated an increase in the oxidation rate of glucose and FAs and a decrease in glycolysis in the heart of normal rats subjected to treadmill running.

Studies on the effect of exercise on metabolism in other organs show diverging findings. Katsumura et al. [35] reported no influence of exercise on the expression of lipid metabolism-related enzymes mRNA in the liver, epididymal adipose tissue and gastrocnemius muscle of high-fat diet-fed mice. High-altitude deer mice acclimatized to hypoxia showed increased muscular capacity for the uptake and oxidation of circulatory glucose during exercise [36]. In the brain of aged rats, exercise improved mitochondrial function without increasing mitochondrial biogenesis [37]. As a response to exercise, skeletal muscles showed increased expression of genes involved in mitochondrial biogenesis, oxidative phosphorylation and FA oxidation through inducing the transcription factor EB [38].

3 Exercise Attenuates Myocardial Lipotoxicity

Free fatty acids (FFAs), the primary energy substrate utilized by the cardiac cells, are supplied via lipolysis of triacylglycerols (TAG) or from blood [39]. The diabetic heart is known to have changes in both glucose and FFAs availability [29]. In diabetes, the intracellular accumulation of TAG and non-esterified fatty acids (NEFA) contributes to apoptosis and build-up of toxic intermediates which result in lipotoxicity. These harmful effects can impair the cardiac function and remodeling in the diabetic myocardium [40, 41].

In type 2 diabetic patients, cardiac dysfunction has been associated with intramyocardial TAG accumulation [42]. In addition, accumulation of TAG and shunting of fatty acids into non-oxidative pathways can trigger accumulation of ceramide and diacylglycerol (DAG). At high concentrations, these intermediates may disrupt insulin signaling [43], induce apoptosis [44] and increase fibrosis of cardiomyocytes through activation of protein kinases (PKCβ) [45].

In normal mice, short-term intensive swim training reduced cardiac levels of ceramide and DAG, and up-regulated diacylglycerol transferase 1 (DGAT1) (TAG

storage enzyme) [46]. Bilet et al. [47] demonstrated that acute bout of exercise increased plasma FFAs levels and cardiac lipid, without hampering systolic function in healthy subjects. On the other hand, endurance training reduced myocardial TAG and improved ejection fraction in obese subjects [48]. However, in obese type 2 diabetic patients, endurance training didn't alter myocardial TAG [49]. A recent study conducted by Hojan et al. [50] showed significant improvement in cardiometabolic markers in prostate cancer men after a trial of a 12-month exercise program. In addition, Mandrup et al. [51] demonstrated reduced risk factors for type 2 DM and cardiovascular disease after high-intensity aerobic training in premenopausal and postmenopausal women. Furthermore, myocardial TAG content has been reduced in diet-induced obese mice subjected to exercise [33]. Of note, acute exercise has been reported to increase the expression of Perilipin-5 (Plin-5) in human skeletal muscle [52]. Plin-5 is believed to facilitate and stabilize lipolysis in the cardiomyocyte and to play a role in the direct transfer of fatty acids between lipid droplets and mitochondria [53]. Plin-5 knockout mice showed resistance to STZ-induced cardiac dysfunction [54]. However, the role of exercise on Plin-5 in the diabetic heart remains to be explored.

4 Exercise Alleviates Cardiac Insulin Signaling and Glucose Metabolism

Type 2 DM is characterized by insulin resistance which manifests the heart and provokes cardiac contractile dysfunction [55]. However, less is known about the mechanisms underlying the impact of insulin resistance on cardiac dysfunction [56]. This poor understanding is attributed to the association of insulin resistance and hyperglycemia with hyperlipidemia, fluctuations in hormones and obesity in the available type 2 DM models. Therefore, teasing out the direct effects of insulin resistance on cardiac function is difficult [57]. Boudina et al. [58] stated that insulin receptor deletion in cardiomyocyte of the CIRKO mouse model would help to trace out the exact impact of insulin resistance on cardiac function.

Diabetes has been reported to preserve the Ras-mitogen-activated protein kinase (Ras/MAPK)-dependent pathway, while impairing the phosphoinositide 3-kinase (PI3K)-mediated pro-survival signaling cascade [59–61], favoring the atherogenic and mitogenic actions of insulin [62]. Through activation of the c-Jun N-terminal kinase (JNK), p38 MAPK and extracellular signal-regulated kinase (ERK) [63, 64], the Ras/MAPK-dependent pathway can promote cell differentiation and apoptosis [65]. On the other hand, impaired phosphorylation of IRS-1 in diabetes and insulin resistance negatively affects the PI3K/PDK1/Akt/aPKC pathway and consequently decreased nitric oxide (NO) bioavailability [66], lipid metabolism [67] and translocation of the glucose transporters (GLUT) -1 and -4 [68].

5 Effect of Exercise on Hyperglycemia-Induced Cellular Pathways in the Myocardium

Hyperglycemia can aggravate cardiovascular dysfunction in diabetes *via* altering different cellular pathways, including PKC pathway, advanced glycation end products (AGEs) pathway, polyol pathway and hexosamine pathway. All of these pathways have a strong potential to increase oxidative stress in the myocardium [69, 70].

5.1 DAG/PKC Pathway

Hyperglycemia increases the synthesis of DAG from glycerol-3-phosphate (G3P), which then triggers activation of PKC pathway in the diabetic myocardium [71]. Activated PKC-β and -δ isoforms inhibit endothelial nitric oxide synthase (eNOS) and NO bioavailability, impair vascular permeability, and induce pro-inflammatory pathway and microvascular matrix remodelling [72–76]. Activated PKC pathway has been reported to induce cardiac hypertrophy, fibrosis and adverse Ca^{2+} handling [77]. In addition, the activity of PKC pathway was associated with reduced cardiac performance [77] and increased reactive oxygen species (ROS) production through activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases [78, 79]. These finding has been supported from studies on type 1 diabetic hearts where pharmacological PKC-β inhibition reduced collagen deposition and preserve the diastolic function [80]. Moreover, transgenic mice overexpressing cardiac PKC-β2 showed increased cardiomyocytes death, dystrophic calcification, cardiac hypertrophy and fibrosis [77].

The role of PKC pathway in exercise-induced improvement of cardiac function in diabetes is not-fully understood. The study of Loganathan et al. [81] showed reduction in myocardial DAG levels following exercise in autoimmune type 1 diabetic rats. However, the reduced DAG in this study was not associated with PKC-β2 both expression and activity.

5.2 Polyol Pathway

Activation of the polyol pathway occurs when the intracellular glucose concentration increases. This pathway is marked by the increased activity of aldose reductase which converts glucose to sorbitol using NADPH as a cofactor. The activity of aldose reductase results in depletion of the intracellular NADPH [82] and can thus impair the myocardial antioxidant capacity [20]. Studies have demonstrated increased activity of aldose reductase in the myocardium of type 1 diabetic mice [83]. Isolated hearts exposed to hyperglycemia showed increased activity of aldose reductase, impaired left ventricular diastolic function and excessive production of

ROS [84]. In addition, activation of the polyol pathway has been reported to predispose the cardiac tissue to ischemic insult. This notion has been supported by the study of Ramasamy et al. [85] where inhibition of aldose reductase protected isolated type 1 diabetic hearts from ischemia reperfusion injury. The impact of exercise on hyperglycemia-induced activation of the polyol pathway in the diabetic myocardium hasn't been investigated yet. Therefore, studies are required to uncover any possible effect of exercise on myocardial aldose reductase.

5.3 AGE/RAGE Axis

In hyperglycemia, intracellular and extracellular proteins and lipids are exposed to high concentrations of glucose and glycolytic intermediates. Proteins, lipids and nucleic acids undergo a non-enzymatic reaction with sugars to produce AGEs [86]. AGEs can alter the elastic properties of blood vessels and modify the extracellular matrix, rendering the tissues less compliant and consequently induce myocardial stiffness [86]. Binding of AGEs to their receptors (RAGEs) on smooth muscle cells, macrophages and endothelium contributes to increased vascular permeability, vasoconstriction, atherogenesis, production of ROS and pro-inflammatory cytokines [87–90], and reduced NO bioavailability [91].

Studies have introduced evidences on the role of AGEs in the development of cardiomyopathy in diabetes [86, 92]. In this context, treatment of STZ-induced diabetic rodents with ALT-711, an AGE cross-link breaker, reduced levels of AGE in the myocardium, improved Ca^{2+} handling, normalize collagen III deposition and attenuated myocardial structural changes [86, 92]. High levels of circulating AGEs have been positively correlated with type 2 diabetes [93] and heart failure [94]. In addition, type 2 diabetic patients exhibited inverse correlation between glycated hemoglobin (HbA1c) and soluble RAGEs (sRAGEs) [95]. This soluble form RAGEs are known to work as scavengers for AGEs [96].

Few studies have demonstrated the effects of exercise on the AGEs/RAGEs axis. Exercise has been reported to increase circulating levels of sRAGEs and reduced cardiometabolic risk factors in type 2 diabetic patients [97]. In aged rats, exercise produced a significant decline in the ventricular AGE levels [98]. In addition, reduction in obesity-induced inflammatory responses and transcription factors in the myocardium [30, 99]. On the contrary, exercise decreased plasma AGEs in obese Zucker rats, whereas exerted no effect on the inflammatory markers [100].

5.4 Hexosamine Pathway

In the hexosamine pathway, the enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT) converts fructose-6-phosphate to glucosamine-6-phosphate (GlcN-6-P). GlcN-6-P is metabolized to produce uridine diphosphate

(UDP)-N-acetylglucosamine which is used by the enzyme O-linked *N*-acetylglucosamine transferase (O-GlcNAc) to modify serine and threonine on cellular proteins [101]. Hyperglycemia induces the expression of O-GlcNAc [102] which was reported to correlate with cardiomyocyte hypertrophy [103], fibrosis, and impaired Ca^{2+} handling [104] and insulin signaling [2].

Exercise was shown to affect the hexosamine pathway in different ways. In lean [105] and STZ-induced diabetic mice [106], long-term intensive swim-training markedly decreased protein O-GlcNAcylation in the heart. On the other hand, treadmill running increased cardiac protein OGlcNAcylation in *db/db* mice [107]. The study of Medford et al. [108] showed very time- and spatial-restricted cardiac protein O-GlcNAcylation in response to acute exercise training. Therefore, further studies are required to explore the exercise-induced changes in cardiac O-GlcNAcylation.

6 Role of ROS in the Progression of Diabetic Cardiomyopathy and Beneficial Effects of Exercise

6.1 Sources of ROS in the Diabetic Myocardium

ROS are generated and degraded during the physiological and homeostatic functions of the living cells [109, 110]. Excessive ROS production leads to oxidative stress and modifications in DNA, lipids, proteins and other cellular molecules [109, 110]. Diabetes and its complications are well-known to be associated with excessive ROS and oxidative stress [109, 110]. Several experimental and clinical studies demonstrated increased oxidative stress in diabetes [111–113].

Oxidative stress mediates the pathogenesis of DCM and increases ischemic susceptibility in the heart of diabetics [115, 116]. Induction of diabetes using STZ led to oxidative stress and abnormal cardiac contraction and relaxation in guinea pigs [117]. In rats with pre-diabetes induced by a single low dose of STZ, diastolic dysfunction, and increased left ventricular mass and wall thickness were recorded [118].

Impaired insulin-mediated glucose uptake, glycogenolysis and gluconeogenesis in peripheral tissues in diabetes lead to hyperglycemia and increased ROS production in the heart [70]. Several pathways are implicated in excessive production of ROS in the diabetic heart, including leakage of the mitochondrial electron transport chain, uncoupling of nitric oxide synthase (eNOS), interaction of AGEs with RAGEs, increased activity of xanthine oxidase, 12/15-lipoxygenase (LOX) and NADPH oxidases [110] (Fig. 12.1).

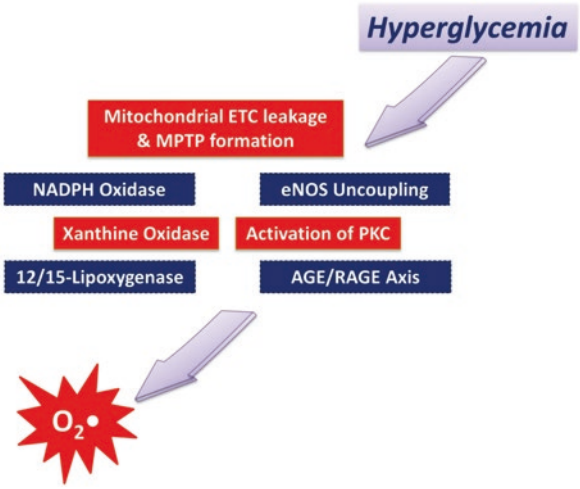

Fig. 12.1 Hyperglycemia-induced superoxide (O_2•) generating pathways. *ETC* electron transport chain, *MPTP* mitochondrial permeability transition pore, *NADPH* nicotinamide adenine dinucleotide phosphate, *AGEs* advanced glycation end-products, *eNOS* endothelial nitric oxide synthase, *PKC* protein kinase C

6.2 *Exercise Attenuates ROS Generation* via *Mitochondrial Electron Leakage*

In oxidative phosphorylation, the transfer of electrons into the electron transport chain located on the inner mitochondrial membrane is directly related to the concentration of intracellular glucose. Under hyperglycemic conditions, the electron transport chain become saturated and electrons are forced to be transferred to oxygen and generates superoxide anions (O_2•) [119] (Fig. 12.2).

Superoxide dismutase (SOD) catalyzes conversion of the generated O_2• to hydrogen peroxide (H_2O_2) which is then decomposed by glutathione peroxidase (GPx) and catalase (CAT). H_2O_2 decomposition can produce hydroxyl radicals (OH•) which are highly damaging. OH• radicals induce the formation of the mitochondrial permeability transition pore (MPTP). In oxidative stress conditions, H^+ passes down the electrochemical gradient through pores in the inner mitochondrial membrane into the mitochondrial matrix without ATP generation. This uncoupling of the electron transport chain leads to further generation of O_2•, swelling of the mitochondrial matrix and leakage of cytochrome C into the cytosol causing apoptosis [120]. Experimental [26, 27, 111] and clinical studies [112] have reported excessive mitochondrial ROS generation which contributes to mitochondrial dysfunction. Mitochondrial ROS can damage membranes and DNA, and impair the activity of electron transport chain, generating more ROS [121]. This notion was supported by the findings of Shen et al. [122] who reported reduced ROS and normalized mitochondrial function via overexpression of the mitochondrial manganese SOD (MnSOD) in the heart.

Regarding the effect of exercise on ROS-induced mitochondrial dysfunction, a transient increase in cardiac ROS has been reported after acute bout of exercise

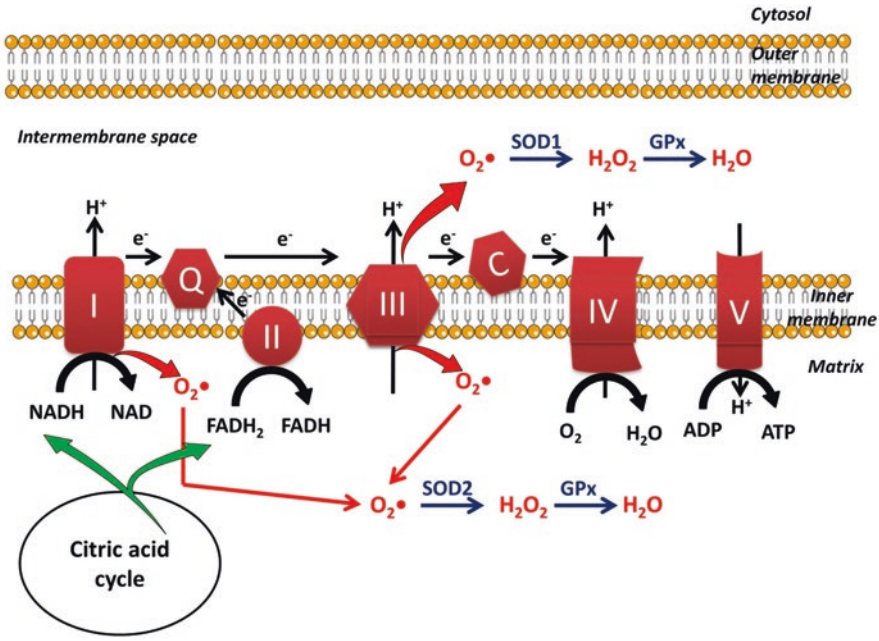

Fig. 12.2 Superoxide (O_2•) production through electron transport chain leakage. Under hyperglycemic conditions, the electron transport chain become saturated and electrons are forced to be transferred to oxygen and generates O_2•. *NAD* nicotinamide adenine dinucleotide, *FAD* flavin adenine dinucleotide, *SOD* superoxide dismutase, *GPx* glutathione peroxidase

[123–125]. Although not fully understood, this transient increase has been suggested to be mediated through rigorous cardiac contractions which increase the flow of electrons through the electron transport chain and formation of O_2•. It seems that this burst of ROS is critical in the cardiac response to exercise. In line with this, the beneficial cardiac responses in rodents [126] as well as health promoting effects of exercise in humans [127] have been impaired on antioxidant therapies. Prolonged acute exercise increases ROS production, uncoupled respiration and mitochondrial membrane potential in mitochondria isolated from rat hearts as described by Bo et al. [124]. Continued exercise resulted in normalization of the mitochondrial ROS levels.

6.3 *Benefits of Exercise on NADPH Oxidase-Dependent ROS Production*

NADPH oxidases (NOXs) represent a major source of ROS in cardiomyocytes [128]. NOXs generate ROS physiologically as a mean of cellular defense against pathogens [129]. NOX2 and NOX4 are the primary cardiac isoforms [130] that

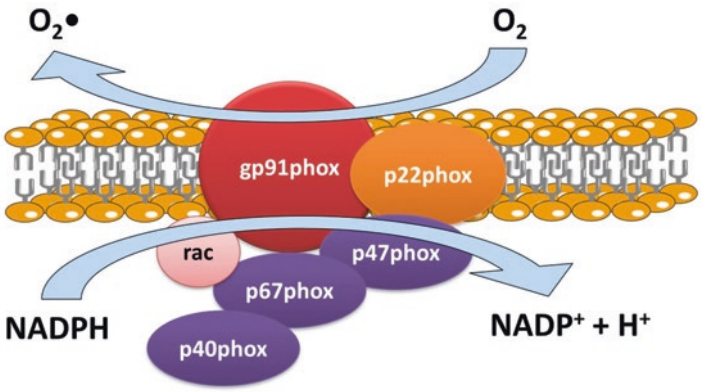

Fig. 12.3 Components of NADPH oxidase

modulates multiple redox-sensitive proteins and signaling pathways [131]. The interaction between cytochrome b558 and the cytosolic components of NADPH oxidase generates O_2• through catalyzing the transfer of electrons to molecular oxygen [110] (Fig. 12.3). Therefore, increased expression and activation of NOXs have been reported in animal models of type 1 and type 2 DM [114, 132] and were associated with the pathogenesis of diabetes-associated vascular disease [133]. In this context, rats with left ventricular hypertrophy (LVH) [134] and human diabetic patients with cardiomyopathy [135] showed increased activity and expression of NOXs. In guinea pig with LVH, NOX-dependent O_2• production and expression of NOX2 were significantly increased in the cardiomyocytes [136]. Overexpression of NOX4 increased O_2• generation, cardiac dysfunction and apoptosis of cardiac cells [137]. Recently, Sharma et al. [138] demonstrated that STZ-induced diabetes in rats significantly increased left ventricular p47phox and p67phox both mRNA and protein expression.

Studies on the genetic and pharmacological inhibition of NOXs have further highlighted the role of these ROS-generating enzymes in mediating cardiomyopathy. In type 1 diabetic mice, the inhibition of NOX2 reduced myocardial fibrosis and improved cardiac function [139]. Treatment of type 2 diabetic rodents with angiotensin receptor blocker reduced NOX2 expression, ROS production and fibrosis of cardiomyocytes [114, 132]. Specific deletion of rac1, a cytosolic component of many NOX isoforms, in cardiomyocytes significantly reduced hyperglycemia-induced myocardial dysfunction, up-regulation of NADPH oxidase activity, ROS generation and cardiomyocyte apoptosis [140]. The hyperglycemic *db/db* mice exhibited marked inhibition of cardiomyocyte NADPH oxidase activity and apoptosis following treatment with a rac1 inhibitor [140]. Collectively, these data show the critical contribution of NADPH oxidase-dependent ROS production in DCM.

The effect of exercise training on the expression and activity of NADPH oxidase in the diabetic myocardium has been demonstrated in several studies. Grijalva et al. [141] reported a significant reduction in NOX2 activity in the myocardium of type

2 diabetic rats after long-term endurance training. In the study of Sharma et al. [138], type 2 diabetic rats underwent a 3 week treadmill endurance exercise protocol exhibited significantly reduced expression of NADPH oxidase subunits. This study added support to the findings of Bidasee et al. [142] and suggested how exercise training improves cardiac function in diabetes. A recent study conducted by Veeranki et al. [143] showed that moderate intensity exercise improved mitochondrial function through restoration of connexin 43 (Cx43) networks and mitochondrial trans-membrane potential in db/db mice.

6.4 *Effect of Exercise on eNOS Uncoupling*

Cardiovascular endothelial function depends on the physiological coupling of eNOS haem group with the substrate L-arginine, using the co-factor tetrahydrobiopterin (BH4) during NO synthesis [144]. Under oxidative stress conditions (Fig. 12.4), BH4 is converted to 7,8-dihydrobiopterin (BH2), which promote uncoupling of eNOS and induce the synthesis of O_2• instead of NO [144]. O_2• can react with NO produced by the activity the inducible form of NOS (iNOS) to form the versatile oxidant peroxynitrite [145]. In diabetes, hyperglycemia favors the expression of iNOS and uncoupling of eNOS leading to increased production of O_2• [146]. The resultant NO bioavailability and formation of peroxynitrite are associated with the progression of DCM and increase in myocardiocytes cell death [147, 148]. The study of Jo et al. [149] showed up-regulation of iNOS, increased levels of the reactive nitrogen species, 4-hydroxy-2-nonenal (4-HNE) and nitrotyrosine, in the heart of diabetic mice.

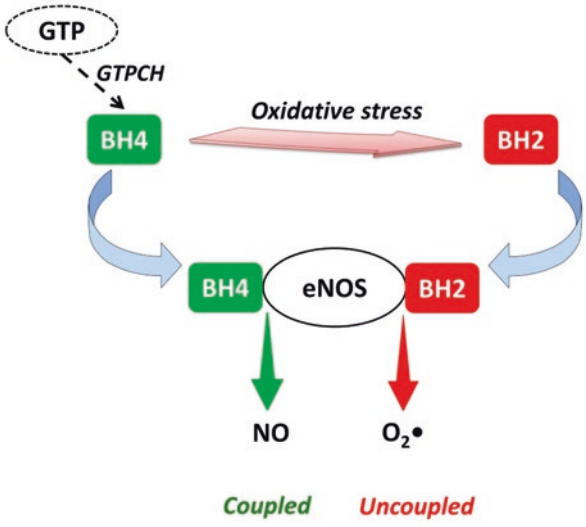

Fig. 12.4 Uncoupling of eNOS and generation of superoxide (O_2•) rather than nitric oxide (NO) under oxidative stress conditions. *eNOS* endothelial nitric oxide synthase, *BH4* tetrahydrobiopterin, *BH2* 7,8-dihydrobiopterin, *GTPCH* GTP-cyclohydrolase-I

The acute effects of exercise on cardiac NOS isoforms in diabetes are not fully understood. However, long-term exercise has been reported to increase NO availability in hearts of health rats [150]. In addition, low-intensity endurance training increased myocardial eNOS expression and NO availability in type 2 diabetic Goto-Kakizaki rat [141]. On the contrary, Kleindienst et al. [151] showed that regular exercise reduce iNOS expression and nitro-oxidative stress in the heart of obese diabetic mice with no effect of β3-adrenergic receptor eNOS pathway.

6.5 Effect of Exercise on Xanthine Oxidase

Xanthine oxidase plays an important role in the pathogenesis of non-diabetic cardiac pathologies; however, its contribution to DCM is not well established [152]. It is an extra-mitochondrial enzyme located in the cytosol of cardiomyocytes and produces H_2O_2 and O_2• during the metabolism of xanthine and hypoxanthine into uric acid [110]. In dogs with induced dilated cardiomyopathy, a fourfold increase in xanthine oxidase mRNA has been reported [153]. In this animal model, xanthine oxidase inhibition resulted in significantly improved myocardial contractility and performance [153]. In *C57/BL6* diabetic mice, inhibition of xanthine oxidase improved cardiac dysfunction by decreasing fibrosis and oxidative/nitrosative stress [154]. In addition, xanthine oxidase inhibition attenuated left ventricular dysfunction in STZ-induced diabetic rats [155]. Till now, nothing in known about the effect of exercise on xanthine oxidase in the diabetic heart. Therefore, studies investigating the effect of acute and long-term exercise on the expression and/or activity of xanthine oxidase in DCM are required.

6.6 Effect of Exercise on 12/15 Lipoxygenase (LOX)

12-LOX and 15-LOX are members of a family of enzymes that oxidatively metabolize arachidonic acid into 12- and 15-hydroxyeicosatetraenoic acids. ROS are released during the metabolism of arachidonic acid by 12- and 15-LOX. Activation of these enzymes has been reported to be induced by hyperglycemia and to be associated with cardiac oxidative stress and DCM [110]. 12/15-LOX-knockout mice with STZ-induced diabetes exhibited reduced cardiac fibrosis when compared with the wild-type mice, suggesting the role of 12/15-LOX in DCM [156]. In addition, deletion of 12/15-LOX in diabetic mice resulted in decreased cardiac lipid peroxidation [156]. These findings suggest that 12/15-LOX inhibition may represent a therapeutic target against DCM. As yet, no studies have investigated the effect of exercise on 12/15-LOX in the diabetic heart.

6.7 *Effects of Exercise on AGEs-Induced ROS*

Glycation is the non-enzymatic covalent bonding to glucose to proteins and lipids. Chronic hyperglycemia induces glycation and cross-linking of the glycation products to produce AGEs. AGEs can bind to RAGE on endothelial cells and macrophages, triggering excessive production of ROS and pro-inflammatory cytokines [157]. The exact mechanism of AGE/RAGE-induced ROS generation in not fully explored; however, evidences suggest an involvement of NADPH oxidase [158, 159]. AGE/RAGE-derived ROS can induce NADPH oxidases and provoke further ROS production [159]. It has been also hypothesized that NADPH oxidase-derived ROS can have similar effect on AGE/RAGE-derived ROS in a positive feedback loop [159].

RAGE-expressing human endothelial cells exposed to AGEs exhibited increased ROS production and expression of tissue factor, pointing to the presence of inflammation [158]. A recent study by Hou et al. [160] showed significantly increased expression of RAGE, nuclear factor-kappa B (NF-κB) and inflammatory cytokines in the myocardium of diabetic rats.

There are currently scarce data available on the effect of exercise on AGEs and RAGE in DCM. However, exercise training has been reported to reduce lipid peroxidation, ROS, activation of NFκB, interleukin-6 (IL-6) and advanced glycation in the aortas of aged rats [161] (Fig. 12.5). This study explained that the

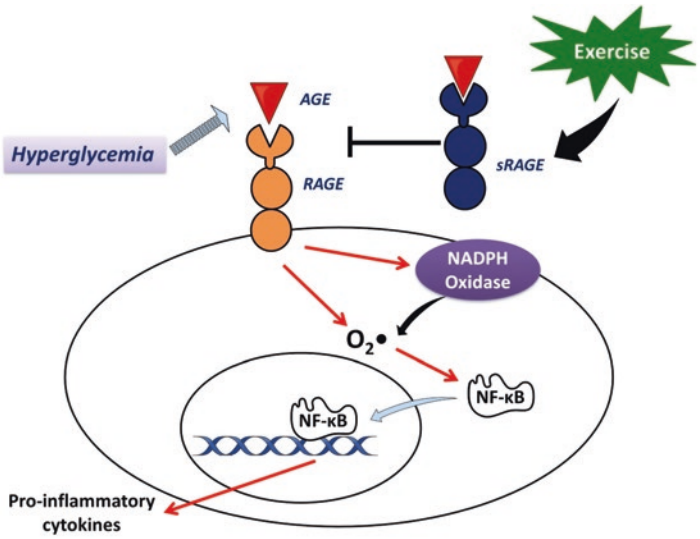

Fig. 12.5 Effect of exercise on hyperglycemia-induced formation of cross-linked AGEs. *RAGE* receptor for advanced glycation endproducts, *AGEs* advanced glycation end-products, *NF-κB* nuclear factor-kappa B, *sRAGE* soluble receptor for advanced glycation endproducts, O_2• superoxide

vasculature-protecting effects of exercise are mediated, at least in part, through suppression of glycation. Another study conducted by Santilli et al. [162] showed the beneficial effects of regular high-intensity exercise training on platelet activation markers, lipid peroxidation and AGE/RAGE axis. Therefore, further studies are required to highlight the effects of exercise training on the AGE/RAGE axis in DCM.

7 Exercise Increases Myocardial Antioxidant Capacity

Impairment of antioxidant defenses is a feature of the diabetic myocardium; therefore, enhancement of the antioxidant system in the heart could exert beneficial effect against DCM. In this context, exercise training has been reported to up-regulate gene and protein expression of the antioxidant defenses in the heart of diabetic and obese animal models [33, 163]. The study of Bo et al. [124] showed increased expression and activity of myocardial MnSOD in response to acute exercise. A recent study showed increased cardiac SOD-2 and catalase expression in response to swimming training in ovariectomized rats [164]. Hyatt et al. [165] demonstrated increased protein levels of SOD-2 in the heart of Sprague-Dawley rats following endurance exercise training. In addition, exercise reduced lipid peroxidation and increased the abundance of antioxidant defenses in cardiac tissues of hypertensive ovariectomized rats undergoing fructose overload [166].

The mechanism behind the beneficial effect of exercise on the myocardium antioxidant defenses involves up-regulation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), a master regulator of cellular antioxidants (Fig. 12.6). Tan et al. [167] reported exercise-induced increase in insulin sensitivity and subsequently Nrf2 activity through the PI3K pathway in myocardial cells both *in vivo* and *in vitro*. To mimic acute exercise, Horie et al. [168] applied electrical pulse stimulation (EPS) in C2CL2 myotubes and showed that the increased expression of Nrf2 and its related antioxidant genes was correlated to intensity and duration of the stimulus. Interestingly, this induced antioxidant gene expression was blunted in response to Nrf2 knockdown. Moreover, mice showed increased Nrf2 gene [169, 170] and protein expression [170], and Nrf2-dependent phase II enzymes [169, 171] following a single bout of acute exercise. Accordingly, exercise up-regulated Nrf2 signaling in mouse myocardium as demonstrated in different studies [125, 172]. Furthermore, Muthusamy et al. [125] reported increased nuclear accumulation of Nrf2 and expression of phase II antioxidants in response to exercise in the myocardium of wild-type mice when compared with $Nrf2^{-/-}$ mice. These data highlight that exercise exerts its benefits through activation of Nrf2 signaling. However, more studies are needed to explore the possible role of exercise on Nrf2 signaling in the diabetic myocardium.

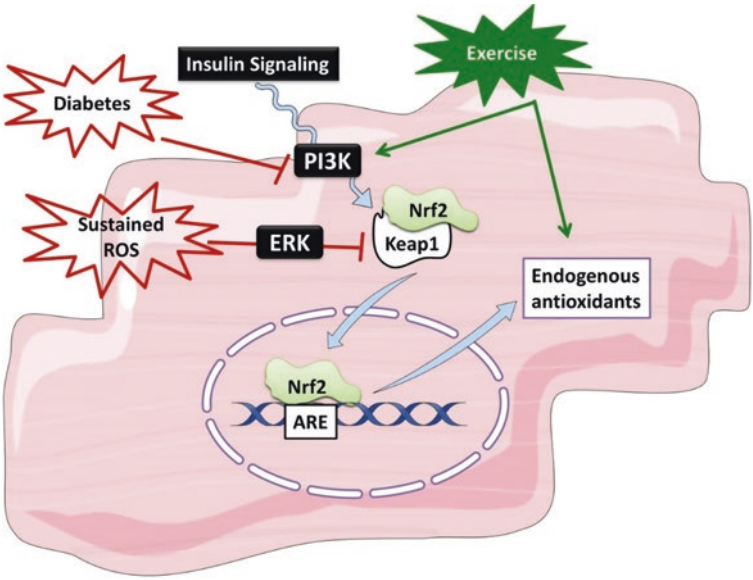

Fig. 12.6 Effects of exercise on Nrf2 and endogenous antioxidant defenses in the diabetic myocardium. Sustained ROS in long-term diabetes impairs insulin signaling, reduces PI3K activity and triggers ERK phosphorylation [20]. *ROS* reactive oxygen species, *Nrf2* nuclear factor erythroid 2 related factor 2, *ARE* antioxidant responsive element, *PI3K* phosphoinositol 3-kinase, *ERK* extracellular signal related kinase

8 Concluding Remarks

Exercise exerts beneficial cardiac effects and influences several metabolic changes associated with diabetes. Different molecular mechanisms are obviously involved in the myocardial responses to exercise. Evidence in the literature advocate that exercise intervention is a potent tool in attenuating diabetic cardiomyopathy. The beneficial effects of exercise on the diabetic myocardium include amendment of the metabolic derangements, lipotoxicity and hyperglycemia-induced ROS-generating pathways. Although studies have demonstrated the effect of exercise on several pathways, other pathways have not yet been investigated. Experimental animal models of diabetes can help in understanding the molecular pathways of the cardiac responses to exercise and to develop new treatment strategies for diabetic cardiomyopathy.

Conflict of Interest None

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Chapter 13
Cardiac Aging – Benefits of Exercise, Nrf2 Activation and Antioxidant Signaling

Madhusudhanan Narasimhan and Namakkal-Soorappan Rajasekaran

Abstract Cardiovascular dysfunction and heart failure associated with aging not only impairs the cardiac function but also the quality of life eventually decreasing the life expectancy of the elderly. Notably, cardiac tissue can prematurely age under certain conditions such as genetic mutation, persistent redox stress and overload, aberrant molecular signaling, DNA damage, telomere attrition, and other pathological insults. While cardiovascular-related morbidity and mortality is on the rise and remains a global health threat, there has been only little to moderate improvements in its medical management. This is due to the fact that the lifestyle changes to molecular mechanisms underlying age-related myocardial structure and functional remodeling are multifactorial and intricately operate at different levels. Along these lines, the intrinsic redox mechanisms and oxidative stress (OS) are widely studied in the myocardium. The accumulation of reactive oxygen species (ROS) with age and the resultant oxidative damage has been shown to increase the susceptibility of the myocardium to multiple complications such as atherosclerosis, hypertension, ischemic heart disease, cardiac myopathy, and heart failure. There has been growing interest in trying to enhance the mechanisms that neutralize the ROS and curtailing OS as a possible anti-aging intervention and as a treatment for age-related disorders. Natural defense system to fight against OS involves a master transcription factor named nuclear erythroid-2-p45-related factor-2 (Nrf2) that regulates several antioxidant genes. Compelling evidence exists on the Nrf2 gain of function through pharmacological interventions in counteracting the oxidative damage and affords cytoprotection in several organs including but not limited to lung, liver, kidney,

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brain, etc. Nevertheless, thus far, only a few studies have described the potential role of Nrf2 and its non-pharmacological induction in cardiac aging. This chapter explores the effects of various modes of exercise on Nrf2 signaling along with its responses and ramifications on the cascade of OS in the aging heart.

Keywords Cardiovascular dysfunction • Aging • Exercise

1 What Is Cardiac Aging?

Any abnormal change that occurs in cardiovascular structure and function below or above the optimal clinical threshold with age is termed as “Cardiac Aging”.

2 What If Cardiac Aging Is Unattended?

Like systemic aging, myocardial aging also occurs ubiquitously and inevitably. Interesting is the fact that each organ can age at different a pace and at times, cardiac aging can be independent of systemic aging. While the aging that results out of natural deterioration of (i) myocardial structure-function, (ii) cardioprotective, and (iii) repair processes is termed as normal or physiological cardiac aging, the pathological cardiac aging refers to the one that is a resultant of non-normative events such as stress, disease and/or any toxic challenges. It is to be noted that the normal myocardial aging does not itself culminate in heart failure. At the same time, the effect of normal (healthy) aging need not always be recognized and differentiated from the effect of pathology, a thought set forth by Sobel [1]. In other words, the two need not be looked independently either where one can aggravate the other. Notably, certain normal age-related changes besides increasing the risk of developing CVD and CHD produces clinical heart complications such as amyloid heart disease, protein aggregation disease, hypertrophic cardiomyopathy, aortic stenosis and several others [2–4].

If both the normal and pathological cardiac aging is overlooked, over time, they can progress and cannot be effectively managed leading to irreversible and complete damage. This can pose economic burden not only at the individual level but also at the societal level as well as the whole nation level. In addition to a decline in the integrity of the cardiovascular system, if unattended, the financial burdens and psychological complications due to disruption in maintaining independence in the daily living of the affected individuals will emerge. This will lead to the feeling of helplessness and other series of emotional disturbances that could cause an indirect aggravating source of the existing cardiac complication leading to irreversible damage in the aged population. Further, the individuals’ physical limitation in the form of heart disease can extend to impact mental health that in turn, can seep into society and burdening the latter in many forms including but not limited to (i) direct health

care costs due to spending on emergency care, hospital care, treatment, rehabilitation etc. for the patients and the dependents (ii) employees requiring additional days off in the workplace leading to lack of productivity, (iii) workplace errors and traffic accidents leading to financial loss (iv) creating a strain in the financing for Medicaid and Medicare that originates from tax dollars.

3 Why Is Cardiac Aging Important and What Does Cardiac Aging Research Mean?

The American Heart Association has estimated that 83.6 million Americans to have been diagnosed with at least one CVD [5]. Among that population, 42.2 million is approximately 60 years of age or older, with the mortality rate of those above the age of 75 being 66% [5]. Given that the elderly population (>65 years) of the United States that numbered ~46.2 million in 2014 is estimated to be more than doubled to 98 million by the year 2060 [6], there is a huge risk in the emergence of a large segment of elderly individuals with age-associated cardiac dysfunction. Another important reason as to why cardiac aging studies are critical is that there are no uniform clinical characteristics of CVD and importantly, the course of CVD varies and increases dramatically with advancing age [7]. Further, many of the physiological alterations in the aging cardiovascular system and the underlying reasons are not yet fully understood and solved. The cardiac aging research means (i) identifying and quantifying potential areas of normative, age-related decline in the cardiac fitness and determining if and how these changes are relevant in the pathological aging process, (ii) address specific gaps in knowledge and construct improvised heart disease knowledge utilizing the information derived from the interplay of aging and what, and how, cardiac risk factors contribute to the development of symptoms/heart failure, and (iii) glean crucial insights to improve the predictive power of the disease and aid the scientific community to devise appropriate intervention and management tools. Thus, relevant and rigorous evidence-based understanding of the aged heart is highly essential not only to inform the clinicians to judiciously manage and treat the failing, senescent hearts but also to extend healthy and independent living of the growing older population.

Although drug-based interventions against cardiac complications in the older people is still a choice, for the group of aged individuals with borderline to mild heart disease risk, due to their age-associated inherent decline in the body metabolic and excretory functions, pharmacological interventions remain the second line of treatment. Instead, lifestyle changes are the preferred choice of the prevention strategy. Abundant data suggest that physical activity in combination with healthy dietary consumptions and avoiding risk factors such as smoking, drinking etc., as a lifestyle change for reducing the risk of cardiovascular disease (CVD) and coronary heart disease (CHD) and improving the heart health [8–10]. In particular, exercise, a term that includes both exercise training and physical activity has been shown to elicit structural and functional benefits to the human cardiovascular system and

reduces the risk of CVD [11, 12]. Further, exercise training and physical activity have been shown to increase the longevity [13–16]. Understandably the effect of exercise varies significantly among different individuals and also in comparison to certain drug based treatments, exercise as an intervention has been shown to exert comparably fewer benefits [17, 18]. This depends on several factors including but not limited to age, type of exercise, environmental conditions in which exercise is being performed, individuals metabolic capacity and several others [19–25]. Importantly, an elaborative prospective study with ~27,000 human subjects indicated that, although significant, only ~40 ~ 60% of the risk reduction of CHD and CVD is contributed by exercise and physical activity-related modification of traditional risk factors such as inflammatory, homeostatic factors, blood pressure, traditional lipids, BMI, HbA1c, homocysteine etc. [26, 27]. Notably, physical inactivity has been identified as the fourth leading risk factor for global mortality by the World Health Organization [28]. Although much remains to be learned as to how and why the exercise exerts differential effects, given the relatively less harmful effects (unless and until appropriate type and the right amount of exercise are performed), the positive benefits of exercise to the cardiovascular system cannot be denied.

Though several investigations have attempted towards understanding the cardiovascular aging and aging-cardiovascular pathway, an apparent translational divide still prevails between the basic mechanistic elucidations and clinical investigations and/or approaches. Growing evidence from basic and clinical studies suggest that an optimal level of endogenous reactive oxygen species and redox signaling pathways govern cardiovascular physiology (Fig. 13.1). Specifically, disrupted redox

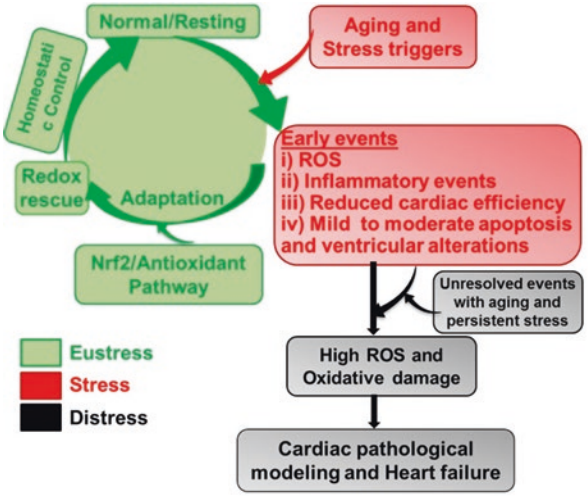

Fig. 13.1 Types of stress and their impact on cardiac health. Aging or any stress initially triggers physiological alterations (representing stress – *red*). In response to oxidative stress, system evokes Nrf2-antioxidant signaling to maintain homeostatic redox and restores the normal function (representing eustress – *green*). Uncontrolled and/or chronic stress conditions impair the defense mechanisms leading to pathological remodeling and cardiac dysfunction (representing distress – *black*)

signaling being a common denominator for aging and several cardiovascular diseases such as heart failure, stroke, myocardial infarction (MI), cardiomyopathies, hypertension, coronary heart disease etc. [29–32], in this chapter, we will discuss on how cellular antioxidant signaling influence cardiac aging and its associated phenotype in the cardiovascular system in response to different types of exercise.

4 Relevance of Redox to Aging and Cardiac Health

Accumulation of evidence indicates that redox state of a cell and their responses to stress plays a critical role in aging and cardiovascular health [29, 30, 32]. In fact, redox signaling has a close relationship to the oldest free radical theory of aging [33] as well as the recently reviewed genetic mutation theory, the wear and tear theory, and the cellular waste accumulation theory [34]. Several clinical and preclinical studies have shown an age-related oxidative shift in the thiol/disulfide redox status, particularly in the ratio of reduced to oxidized glutathione, which is a major cellular antioxidant [35–37]. Further, age-related oxidative stress can inactivate the conformation, stability, molecular interactions, and activity of several signal transducers such as phosphatases, ion transporters, receptors, kinases that participate in diverse processes namely gene transcription, proteasome inactivation, loss of repair mechanisms etc., suggesting that there is an unavoidable alteration in the most, if not all, of the physiological mechanisms and set points concerning the redox homeostasis [38–40]. This can sustain the aging-oxidative stress cyclical process. At the same time, it is also to be reminded that the oxyradicals or oxidants may not always cause damage and perturb the aging process. In other words, the redox signaling cannot be completely viewed as a mechanism similar to on-off switch (presence of oxidants/absence of oxidants), rather it must be viewed as a specific and/or precise balancing of the oxidation-reduction process and the levels of redox messengers lying within the physiological range [41]. However, an ambiguity in the field still exists as to whether or not redox stress plays an important role in the aging process [42, 43]. But, given the fact that oxygen is essential for life with an accrual and increased rate of oxidative damage and its associated physiological impairments with age, it is not surprising to believe that any exogenous or endogenous mechanisms that alter the oxygen metabolism perturbing the redox homeostasis either largely or continuously can derange the health maintenance processes and can impact both the longevity as well as the quality of aging [44–46].

Effective heart function also chiefly depends on the oxidative energy production which is evident from the fact that it consumes approximately 8–15 ml O_2/min/100 g tissue at resting state. While this can be increased to >70 ml O2/min/100 g myocardial tissue during vigorous exercise [47, 48]. In short, heart exhibits high oxygen consumption. Further, the heart is composed of array of cell types such as cardiomyocytes (essential for heart contraction contributes by generating and conduct electrical signals), fibroblasts (ensures proper cardiac form and cell-cell communication), endothelial cells (functions in nutrient intake, oxygen

transport, maintenance of barrier function and permeability), smooth muscle cells (responsible for peripheral resistance to blood flow generated by the beating heart and regulation of blood pressure), cardiac mast cells (store and release a variety of biologically active mediators), cardiac macrophages (functioning in remodeling, wound healing and regeneration) [43, 49–54], all of which rely on redox reactions and redox signaling messengers to send physiological inputs to perform these fundamental processes.

While, the redox homeostasis is a precise balance between the endogenous levels of oxidants and antioxidants, in today's situation our human system is persistently challenged to maintain it due to continuous fluctuations in the environment, dietary styles, exposure to toxic chemicals coupled with less physical activity, chronic stress and other unhealthy lifestyle choices. In addition, heart being a vital organ abundant in mitochondria, a major site of reactive oxygen species (ROS) production, the processes involving oxygen could generate highly reactive oxyradicals within the cardiac cells and this when uncontrolled can cause an imbalance in the redox equilibrium towards oxidant that can have far-reaching consequences on the basic metabolic processes. This can mount oxidative stress (OS) driving the processes related to oxidative damage eventually leading to cardiac dysfunction and heart failure [55, 56]. Loss of redox control within the cell and the resultant OS can disrupt numerous physiological functions including but not limited to gene expression, cell survival/apoptosis, cardiomyocyte differentiation, impaired functions of enzymes, proteins and transcription factors, excitation-contraction coupling of heart, regulation of blood flow etc., and perturb cellular integrity and organ homeostasis [55–60]. These effects vary in magnitude depending on the cellular context and the extent of redox disturbance. It has long been recognized that an acute toxic and chronic "disruption of oxygen metabolism and redox regulation" is a crucial determinant to major cardiovascular problems, if left untreated, the disease can advance to chronic phase and cause multi-organ failure eventually proving fatal [61]. In short, the obligatory redox mechanisms crucial for basic life-sustaining processes can turn into devastating life-events.

Recent epidemiological studies indicate an increased incidence of CVD among the aged population (>65 years) in the United States in the past years [5, 62–64]. Strikingly, the recent health status of the United States compiled by Centers for Disease Control and Prevention's (CDC) National Center for Health Statistics (NCHS) reports that mortality due to cardiac diseases occupy 5th, 3rd, 2nd and 1st spot in the age group <25, 25–44, 45–64 and over 65 years of age, respectively (CDC-National Center for Health Statistics, 2015). This provides a compelling point that the diseases of the heart has a strong correlation with age and is the number one cause of death in the elderly population. Relevant to these facts, an uncontrolled OS has been associated strongly with the etiology of stroke, coronary heart disease, ischemia/reperfusion injury, atherosclerosis, and hypertension [57, 65–67]. Further, due to inherent nature of the heart cells to undergo limited mitosis coupled with the attrition of biosynthetic processes and increased oxidative burden in aging, the ability of the heart to sustain its structure-function relationships and keeping up the cardiac performance can be remarkably affected. Thus, age-associated OS has been regarded as an independent factor to profoundly impact CVD and heart failure [55, 68, 69].

Notably, cardiac aging in the murine model is highly consistent with the age-related cardiac changes observed in a healthy human population [70]. Indeed, histopathological, echocardiographic and signaling studies indicate that the murine heart undergoes subendocardial, interstitial fibrotic changes, amyloid deposition along with hypertrophy, diastolic dysfunction, reduced functional reserve and molecular alterations with age parallels that of elderly humans [70–75]. Like other tissues, a human heart also has the replicative potential [76, 77] and thus myocardial aging can also be accompanied by an imbalance between the reduction of myocyte growth and death. Along these lines, an interesting study demonstrated that the endomyocardial biopsies from patients with heart failure revealed shortened telomeres, increased cellular senescence and cell death [78]. In addition, telomeric length in circulating leukocytes has been shown to be a representative of that in the cardiac myocytes and role of oxidant stress in telomere attrition in this context is well known [79–81]. Using telomerase ablation, researchers were also able to generate a premature aging animal model that was characterized by an enhanced apoptotic myocyte death along with poor myocyte growth [82]. Interestingly, lack of telomerase and the subsequent telomere erosion also led to increased oxidative stress in cardiomyocytes [83] thus hinting at the fact that the redox-telomere axis can operate in a loop perpetuating each other in the myocardium. Further, the redox perturbations associated with an aged heart can particularly induce a functional loss of either cardiomyocyte and/or the supporting cells eventually accruing and/or eliminating those from the system [84–86]. Very recently, under steady state conditions, physiological aging is shown to be accompanied by shifts in the composition of heart-associated leukocyte populations has been reported [87]. Further, the same study demonstrated that heart-directed immune responses along with myocardial functional and structural alterations may spontaneously occur in the elderly, without the presence of any apparent tissue damage or infection. These shifts in the cardiac-resident cells can influence the local milieu modulating several events and affecting the heart regenerative capacity with age progression [78, 88, 89]. At this juncture, it is noteworthy that the age-accompanied oxidative changes and certain clinical manifestations observed in humans are also recapitulated in the myocardium of murine models [70, 90–93]. Thus, the more we appreciate and comprehend the redox control and environment, the better we will understand the biology of the heart and thus on the crosstalk of aging and cardiovascular health.

5 Response to Redox Stress – Nrf2 Signaling and Relevance to Cardiac Aging

In general, the cellular system is inherently endowed with several antioxidants based counteracting mechanisms to respond to and balance the increase in oxidative burden. They either individually or in combination eliminate the free radicals, scavenge and neutralize ROS and their precursor, inhibit ROS generation or sequester the redox-active metal ions required to catalyze the Fenton-type ROS generating

reactions [94]. These antioxidant factors are supplied to the human system either by endogenous process or through the dietary sources. Endogenously, the activation of several signaling proteins and transcription factors such as NFκB, AP1, HIF1α, p53 and Nrf2 (nuclear factor (erythroid-derived 2)-like 2) exerts transcriptional control of antioxidant signaling by regulating the expression of genes that encode antioxidant enzymes, phase-2 enzymes, protein chaperones, and other cytoprotective machinery [95, 96]. Although antioxidants protect the system from the attack of oxidative stress and associated damage, the hypothesis that organismal lifespan can be enhanced by increasing antioxidant defenses is still vague due to conflicting results in certain aging models. For instance, studies in mammals in which levels of endogenous antioxidant genes are experimentally increased have shown that maximum longevity either is minimally increased or not affected [43, 97, 98]. In fact, double transgenic mice overexpressing both CuZnSOD and catalase did not show a significant change in the lifespan [43]. These lifespan studies understandably used the survival of the organism as their endpoint measurement and the possibility that overexpression of these antioxidant genes in slowing down the rate of aging of individual organs are not focused. At this juncture, it is to be considered that certain intrinsic changes always co-occur with age, however, all organs need not age alike and a specific organ can show signs of aging phenotypes before the other. Particularly, organism and organ age need not always proceed concomitantly [99–101]. Especially, this can be true in the case of heart as this is a nonstop working organ with remarkable plasticity besides facing a continuous challenge to its organ reserve, an ability to adjust and function beyond the typical needs. A recent large-scale study revealed that how cellular proteins (that determines the function) age differently in different niches indicating that the cellular property and the physiology of a specific organ can drive its course of aging [102]. Thus, the amplitude of response can vary depending on the severity of oxidant/antioxidant imbalance, inherent tolerance to alterations, cellular context and physiology of the specific organ and several other regulatory factors resulting in either adaptation/advantage (Eustress), stress and/or unresolved stress (Distress or Destruction) [103] (Fig. 13.2).

It is widely agreed that with age there is an apparent decline in the production and activity of biological antioxidants resulting in an overburden of ROS/RNS [56]. A component of the cellular antioxidant and detoxification pathway is the battery of genes bearing the antioxidant response element (AREs) such as NAD(P)H-quinone oxidase-1 (NQO1), heme oxygenase (HO1), ′Ɣ-glutamyl cysteine ligase-catalytic (GCLC), ′Ɣ-glutamyl ligase-modulatory (GCLM), glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase-1 (GPX1), glutathione peroxidase-2 (GPX2), glutathione reductase (GSR), catalase (CAT) to scavenge the endogenous reactive intermediates and toxin export genes (multidrug response transporter family, MDR) [96, 104, 105], all of which are regulated by nuclear factor erythroid-2-p45-related factor-2 (Nrf2). It is originally discovered as a Cap ‘n’ Collar (CNC) family of basic leucine zipper (bZip)-DNA binding protein that can bind to erythroid transcription factor 2 (NF-E2) binding motif [106]. Later it has been established as a major transcription factor that heterodimerizes with small musculoaponeurotic fibrosarcoma (Maf) proteins and drives the expression of

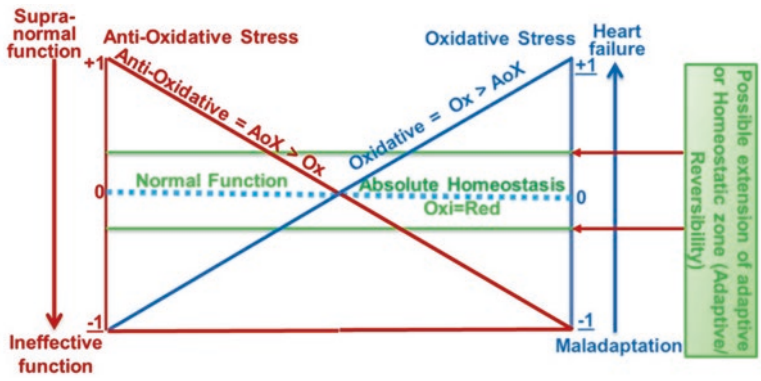

Fig. 13.2 Redox-dependent regulation of cardiac function. Oxidant to antioxidant balance is crucial for optimal cardiac function. The decrease in either oxidants or antioxidants leads to ineffective cardiac function. Abnormal increase of either will result in supra-normal (pathological) function and heart failure

several genes containing the antioxidant response element (ARE) binding motif due to the high similarity of consensus sequence between them [106–108]. Apart from the classical antioxidant targets, Nrf2 regulates genes involved in anti-inflammatory, Autophagy, Proteasomal pathway [109–111].

Nrf2 is regulated by several mechanisms. Under normal redox environment, Nrf2 protein is sequestered in the cytoplasm by its inhibitor protein, Kelch-like ECH associating protein 1 (Keap1) in a hinge and latch model and is targeted for Cullin 3 /Ring-box 1 protein (Cul3/Rbx1)-based ubiquitination and degradation [112–115]. However, upon conditions of oxidative stress or electrophilic stress, Keap1 becomes oxidized at critical cysteine residues inducing a conformational change resulting in dissociation of Nrf2 from Keap1. Subsequently, Nrf2 translocates to the nucleus, partners with Maf and additional proteins, binds to *cis*-acting ARE sequence and promotes gene transcription. In addition to the classical Keap1 based control, the stability of Nrf2 is regulated by E3 ubiquitin ligase based degradation by Skp, Cullin, F-box/β-transducin repeat-containing protein (SCF/β-TrCP) complex independent of Keap1 [116]. Particularly, this has been shown to be mediated by glycogen synthase kinase 3 (GSK-3) that phosphorylates specific serine residues in the Neh6 domain of Nrf2 corresponding to the β-TrCP recognition motif and directs it for degradation [116]. Further, Nrf2 activation may occur following its phosphorylation by several kinases such as mitogen activated protein kinase, phosphatidylinositol 3-kinase, protein kinase C, and protein kinase RNA-like endoplasmic reticulum kinase (PERK) [117, 118]. In a feed-forward manner, Nrf2 protein transcriptionally regulates its own gene expression by binding to two ARE-like motifs that are present in its promoter region [119]. Nrf2 is also regulated by the epigenetics and miRNA-based post-transcriptional mechanisms [120, 121].

Nrf2 possess an evolutionarily conserved role in protection against OS [122]. Of note, the intensive function and relatively minimal and slow rate of replacement of

cardiomyocytes can make the myocardium more susceptible to stress, aging, diseases and other toxic insults [123–125]. A mild to moderate increase in ROS level is shown to activate Nrf2 and subsequent antioxidant and detoxification mechanisms in the vasculature of young animals as an adaptive response [64, 126]. In contrast, under circumstances of strong oxidation as in a 5-month chronic hyperglycemia mice model, Nrf2 and its response in the heart is found to be severely dampened compared to a 2-month old hyperglycemic model that can represent a modest redox perturbation [127]. Notably, nuclear Nrf2 levels were significantly decreased in the autopsied heart specimens from diabetic patients compared to control hearts [127]. This indicates that the intracellular level of ROS in the heart, i.e. low to mild representing physiological and strong to the persistent representing pathological scenario can bi-modally regulate Nrf2 thus making it as an attractive candidate for further studies.

Previous reports suggest that aged mice display a similar reduction in cellular redox capacity as that of Nrf2 knockout mice [118, 128]. In contrast, augmented activity of Nrf2 and ARE-responsive genes has been observed in long-lived rodents such as naked mole rats and Snell Dwarf mice [129–131]. Interestingly a classical study that compared eight rodent species with vastly differing longevities ranging from 4 to 31 years observed a strong correlation between Nrf2-ARE binding activity and maximum lifespan potential [130]. The role of Nrf2 in aging and human disease is extensively reviewed elsewhere [132]. Several mechanistic studies have elaborately demonstrated that age-related deficiency and/or insufficient activity of Nrf2 impairs cell's ability to mount an adaptive response and detoxify the oxygen radicals affecting the redox homeostasis leading to OS and/or oxidant sensitivity in the myocardium and heart failure [56, 69, 126, 133, 134]. Moreover, disturbances in redox homeostasis are reported to induce apoptosis and/or necrosis of myocyte resulting in decreased myocyte number, a hallmark of aging heart that in turn, results in remodeling and hypertrophy [66]. Thus, an increase in ROS concentration that can stem from a combination of two intrinsic sources namely aging and myocardium, an organ that is rich in mitochondria which are a seat of oxidative metabolism has the high likelihood of weakening the heart-directed stress responses in the elderly even in the absence of any pathology. While, in the presence of any pathology, the ability of the cells to respond and restore from toxic challenges could be greatly debilitated. In this connection, such a mechanism involving Nrf2 to control the oxidative burden in the aging heart can be highly relevant. Of note, these lines of evidence and thoughts clearly point out that Nrf2 pathway is at the intersection of both aging and cardiac physiology wherein, a decline in Nrf2-antioxidant signaling during aging can predispose the cardiac tissues to various adverse etiologies of disease development [56, 67]. Taking these facts into account, Nrf2 may be regarded as a "gatekeeper of cardiac longevity and myocardial health".

More complex, yet interesting is the fact that in physically fit who undergoes progressive training, there is a decreased incidence of OS based pathologies. This is due to the reinforcement of adaptive resistance to OS along with induction of trophic factors and activation of oxidative-damage repairing systems [135–140]. Hence, in the next section we will discuss the systematic functions (up and down

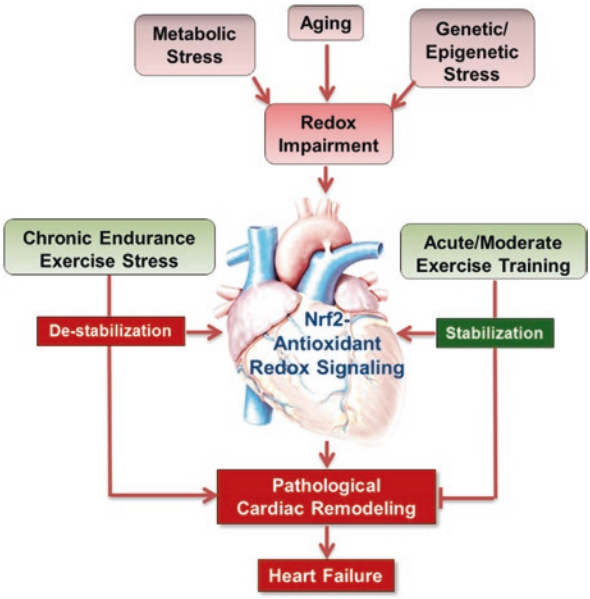

Fig. 13.3 Effects of metabolic and physical stresses on patho(physio)logical cardiac aging. Metabolic and chronic conditions such as obesity, diabetes, hypertension, inflammation, etc. promote hyper-oxidative condition, and structural and functional (systolic/diastolic) remodeling leads to accelerating aging of the myocardium. While endurance exercise stress exacerbates oxidative stress and cardiac remodeling, acute or moderate exercise training preserves cardiac health and prevents remodeling by maintaining myocardial defense system through stabilizing Nrf2-antioxidant signaling

regulation) of Nrf2 with respect to different mode of exercises such as acute exercise stress (AES), endurance exercise stress (EES) and moderate exercise training (MET), and outline the importance of 'OPTIMAL' Nrf2 sustenance for the healthy regulation of redox homeostasis under diverse challenges in the cardiac system (Fig. 13.3). Different modes exercise protocols and their effects are summarized in Table 13.1.

6 Benefits of Acute Exercise on Nrf2 Antioxidant Signaling

Exercise is recognized for maximal oxygen uptake by tissues to elevate and improve the metabolism, thereby enhance myocardial fitness [141]. However, the expansion of cardiac fitness correlates well with the degree of the physical action [142]. In examining the underlying mechanisms for exercise-induced benefits in myocardial health, it has been demonstrated that ROS can activate redox-sensitive transcription factors, including Nrf2, NF-κB and strengthen the antioxidant signaling [96, 120, 143–146]. Prior studies indicated that impairment of the Nrf2-related antioxidant

Table 13.1 Different modes of exercise and their effects on cardiac health in rodents vs. human

Model	Description	Protocol	Effects of myocardium in rodents (animal models)		References (animal models)	Relevance to Human	References (Human)
			Young	Old			
Acute (>1 to <7 days)	Moderate	0–7° angle; 10–15 m/min; 45–60 min/day	Activates Nrf2 signaling, reduces oxidative stress and protects the myocardium		N/A	N/A	
	Endurance	8–12° (uphill) angle; 20–35 m/min; 60–90 min/day	Activates Nrf2-antioxidant signaling	Null response or impaired Nrf2 signaling	[96, 146, 145]		
Chronic (above 2 weeks)	Moderate	0–7° angle; 10–15 m/min; 45–60 min/day	Activates Nrf2- antioxidant signaling, prevents oxidative stress and heart failure	Stabilizes Nrf2- antioxidant signaling, prevents oxidative stress and heart failure	[75, 161, 160, 166, 162]	Increased physical activity including regular walking, is associated with reduced risk for cardiovascular events	[153, 152]; [163]; [164, 165]
	Endurance	8–12° (uphill) angle; 20–35 m/min; 60–90 min/day	N/A	Destabilizes Nrf2 signaling, induces oxidative stress mediated myocardial remodeling and dysfunction	[155, 75, 193]	Cardiac adaptations including hypertrophic cardiomyopathy, and coronary artery anomalies	[191, 192, 153]

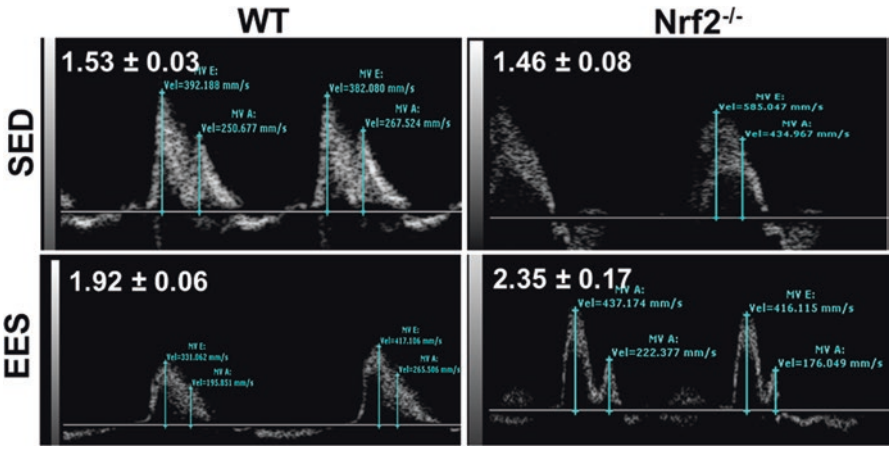

Fig. 13.4 Chronic endurance exercise induces diastolic dysfunction. Mitral valve inflow measurements using pulse wave Doppler (Visual Sonics, Vevo2100 Echocardiography Imager) illustrates the prolonged endurance exercise cause diastolic dysfunction in WT or Nrf2$^{-/-}$ mice on aging

defense mechanisms leads to reduced cardiac performance, dysfunction, fibrotic remodeling, and inflammation through sustained oxidative stress [75, 133, 147]. Accordingly, our previous study demonstrated that acute exercise training stimulated nuclear translocation of Nrf2 and transcriptional activation of its target antioxidants in the heart of WT, while the Nrf2 ablated mice suffered from severe OS upon acute exercise [96]. Overall findings reveal that exercise may exert a beneficial effect in protecting the myocardium through Nrf2-dependent EpRE/ARE signaling pathway, but benefits to exercise differ with age. Aged individuals display several cardiac structural and functional impairments and are increasingly vulnerable to develop pathological remodeling relative to younger ones.

Age-related diminution in myocardial performance and tolerance to exercise are known [2] with the peak maximal oxygen capacity (VO_2) impairs either in the normal or highly active healthy but older people influencing the cardio-respiratory health [148–153]. Recent reports indicate that the physiological level of Nrf2 and its transactivation ability is reduced in aging [75, 154]. An earlier report from our laboratory has shown that the aging mice develop maladaptive oxidative stress and diastolic dysfunction due to exhaustion of existing antioxidant pool to overcome the endurance stress (Fig. 13.4) [155, 156]. Studies in healthy older men indicated a reduced response to acute exercise due to decreased β-adrenergic responses [157]. Recent reports on β1-adrenergic mediated Nrf2/HO-1/HMGB1 axis demonstrated hypoxia/reoxygenation (H/R)- injury in neonatal rat cardiomyocytes [158]. Future investigation on the cross-talk between Nrf2 and β-adrenergic signaling pathways in acute exercise training during aging would be fascinating.

7 Moderate Exercise Training (MET) and Stabilization of Nrf2-Antioxidant Signaling in the Myocardium

Given the role of Nrf2 during short sub-maximal (acute) and maximal endurance stress in aging animals, a moderate exercise training (MET) for a longer duration (6 weeks) seems to significantly augment nuclear Nrf2 level in both the young and aging heart. Subsequent to MET-induced Nrf2 stabilization, an increase in the transcript and protein levels of major Nrf2-targets (i.e. glutathione reductase, hemoxygenase-1, glucose 6-phosphate dehydrogenase and glutamyl cysteine ligase) was also demonstrated [75]. Prior studies have established that a short- and long-term MET promoted myocardial perfusion and functional capacity in animals and humans with ischemic heart disease, and heart failure [152, 156, 159–166]. Notably, MET has been beneficial in preventing age-related cardiovascular diseases [15, 167]. We along with others have shown that, although there was an MET-induced protection seen in aged animals the magnitude of benefits was not as strong as that noted in the younger group [75, 168]. However, the precise mechanisms are still elusive. Based on our previous study, it can be agreed upon that moderate exercise training in aging animals can escalate Nrf2 signaling and restore redox homeostasis [75], but the inherently high oxidative burden that exists during aging could require a much stronger Nrf2 activation and its associated antioxidant signaling than that is currently offered by MET. In other words, optimal benefits of MET could depend on the optimization of redox and this could be one of the crucial points where the young and old diverge with respect to MET's effect. Further, an interesting finding has demonstrated that when animals were subjected to swimming in warm water at 30–32 °C with a duration of 60 min (5 days/week) for 10 weeks that represents MET, showed neovascularization along with improved structure and function of the cardiovascular system [169–171]. Although MET can enhance Nrf2 signaling in the aging model, how far MET can be effective in improving the proangiogenic process in aging is unclear currently. Since, it has been reported that Nrf2 can activate HIF-1α/VEGF pathway [172, 173], investigating the molecular cross-talk between Nrf2 with angiogenesis in response to MET in young and aged hearts could also be worthwhile to narrow the gap in our understanding along these lines. Nevertheless, moderate exercise training is still the most preferred and applied training modality for improving cardiac fitness. However, new training modes and/or strategies are in need for the aged segment. With the current understanding, we suppose an in-depth animal study involving a combination of MET along with a titrated nutraceutical or dietary mode of Nrf2 activation through Protandim or Broccoli, that contains sulforaphane (an activator of Nrf2) respectively will be an ideal starting point. And, hopefully, more studies in this line of thought can provide impetus to explore an effective strategy to improve the cardiac fitness in the elderly.

8 Endurance Exercise Impairs Nrf2-Antioxidant Signaling in the Aging Heart

The strategy of endurance exercise has been proposed to mitigate the onset of sarcopenia with age [174] and sustain the mitochondrial function in aging and related comorbidities [175]. In addition, endurance training has been shown to exert anti-inflammatory effects, increase insulin sensitivity and counteracts the loss of skeletal muscle mass and strength [176–179]. Although, endurance exercise is considered to be a feasible and effective method in older adults [180–182], a continued elevation of oxidative damage of proteins associated with an inability to improve skeletal muscle and mitochondrial protein quality is seen in older people after 8 weeks of endurance training aging [183]. Further, a randomized, single-blinded clinical trial demonstrated that endurance exercise was either 'neutral' or 'negative' with an unaltered rate of oxygen consumption (VO_2), left ventricular (LV) structure and function [184, 185]. Given the inconsistent effects of endurance training besides the duration and intensity of exercise regimen, we also observed that endurance exercise can evoke stress that leads to a hyper-oxidative condition in the heart of aged mice [75]. When the young-adult (~6 months old) mice were stressed to their endurance capacity, an activation of Nrf2 signaling along with augmented myocardial antioxidant response was detected. In contrast, the old mice exhibited a significant decline in Nrf2 and downregulation of its target genes after EES [75]. In particular, the Nrf2 targets such as Nqo1 and Ho1 along with the genes encoding the subunits of γ-glutamyl cysteine ligase (Gcl – Gclm and Gclc), the rate-limiting enzyme for GSH biosynthesis, were significantly decreased in the heart of aging when compared to young mice following EES. Gene expression levels for the ROS scavenging enzymes such as *Sod2*, *catalase* and *Gpx1* were also blunted in the old mice. Induction of mRNA levels for G6PD and GSR, key enzymes responsible for recycling oxidized glutathione (GSSG) back into its reduced form (GSH), revealed a parallel trend, being increased in young, but blunted in old mice following EES. Comparable protein levels of antioxidant enzymes correlated with their transcript levels indicating a tight regulation of Nrf2 signaling, which is diminished in the aging heart after EES. These results indicate that aged hearts were unable to combat EES-induced oxidative stress and hence become susceptible to pathological remodeling. Further, our data using genetically modified Nrf2 mice indicate that a loss of Nrf2 signaling could have detrimental effects besides antioxidant regulation in that it might either directly or indirectly be involved in pathological remodeling of myocardial structure and functional disintegration of the heart in response to intense endurance exercise training (Figs. 13.3 and 13.4). This denotes that a discrete Nrf2 gene content (either presence or absence) might significantly influence the outcome of the endurance training. Consequently, these findings from our laboratory underscores that there might be persisting effects and/or burden of age on Nrf2 dependent redox mechanisms in the heart that can increase cardiovascular disease risk upon endurance training thereby affecting the quality of life, despite the

endurance training is considered to be safe in aging and regarded as a countermeasure for aging [186].

Typically, endurance exercise can increase the physiological demands of the heart and of note, a meta-analysis from 23 studies comprising a total of 294 cases reported that following endurance exercise, there was a 2% reduction in left ventricular ejection fraction (LVEF) transiently [187]. Strikingly, a small change of 1% reduction in LVEF has been shown to increase the risk of fatal and nonfatal cardiovascular events in asymptomatic dialysis patients [188]. Diminution of right ventricle function is also observed with endurance exercise [189]. In another study, rats subjected to long term treadmill running protocol for 18 weeks (2 weeks of progressive training +16 weeks of steady state 1-hour running), a time approximately translates to 10 years of endurance exercise training in humans, exhibited eccentric myocardial hypertrophy, diastolic dysfunction, atrial dilation, together with collagen deposition and higher fibrotic marker expression in both atria and right ventricle [153, 190–193]. These morphological and functional changes are essentially a close mimic of the "athlete's heart" as described in humans. Thus, given the increasing trend of endurance exercise at an alarming rate over the past decades [194, 195], there is an immediate need for detailed understanding of endurance training in the context of the myocardium. Further, since it was reported that an acute reduction in LVEF and myocardial dysfunction could follow a prolonged strenuous exercise due to increased oxidative signaling [196, 197], the relevance of endurance exercise to Nrf2-antioxidant signaling which is intrinsically disrupted in the aged myocardium can be highly important from the standpoint of cardiovascular fitness. More detailed studies with different aged animals spawning 6–24 months subjected to endurance training (mild, moderate, intense) over prolonged period of time in the presence and absence of Nrf2 will comprehensively determine the impact of endurance exercise stress on normal and forced (redox stressed) cardiac aging.

9 Conclusions and Perspectives

Overall, the central theme presented here suggests that Nrf2 is crucial to avail benefits of exercise. Discrete modes of exercise result in a differing degree of favorable effects on cardiac health. Considerable evidence indicates that the cellular redox status and signaling *per se* can shift a physiological adaptation to the pathological event and *vice-versa* in response to any exercise training. Specifically, endurance exercise stress may provoke cardiac exertion and instability in the aged animals. Since Nrf2 and its function progressively declines over age, within a specific segment (age group), for instance, older segment, distinct individuals will likely possess varying levels of redox control. In addition, no two individuals will present themselves with stress levels to a similar extent. Relevant to the real time setting, in certain healthy individuals with lower Nrf2 levels and antioxidant threshold who do not manifest any apparent symptoms, an abstinence of competitive exercise may be required. Further, exercise and Nrf2-dependent redox alterations either individually or in

combination can affect catecholamine regulation that when inappropriately managed, can result in improper calcium handling leading to negative exercise response. Thus, an exercise intervention and its effect must be viewed contextually and more studies focusing on the adverse effects must be welcomed in the future to obtain a complete picture. At the same time, we do not propose that an incomplete picture of redox status as a definite detractor of existing intervention protocol. Considerations to these subtle yet important mechanistic details could confer better outcome with respect to cardiovascular adaptations and avoid any possible risks in the asymptomatic aged group. Nonetheless, to put more prosaically and simply, "Manage Nrf2, Balance the redox, Exercise smarter, Build a healthy heart".

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Chapter 14
Cardiac Fibrosis: The Beneficial Effects of Exercise in Cardiac Fibrosis

Jan Kyselovič and John J. Leddy

Abstract Numerous scientific findings have concluded that individuals who are active tend to develop less cardiovascular disease than those who enjoy more sedentary lifestyles. Animal models have further demonstrated that the beneficial effects of training on the heart effects of training are related to the signaling pathways of myocardial hypertrophy and fibrosis. As such, fibroblasts represent a very important population of cells within the myocardium as they play a crucial role in both cardiac development and response to injury. Fibroblasts establish and maintain the biochemical, electrical and mechanical environment of the heart through their complex interactions with cardiomyocytes. Cardiac injury disrupts the balance between fibroblasts and cardiomyocytes and creates a state favouring inflammation and fibrosis. Although this adaptive response initially serves to increase wound healing, it may eventually lead to increased cardiac damage and cardiac failure if homeostasis is not restored. Myofibroblasts are mediators of both the adaptive and maladaptive components of this reaction. This review focuses on the beneficial effects of exercise in cardiac fibrosis as demonstrated in basic research studies. Attention will be given to the characterisation of the relationship between exercise and cardiac remodelling, including the cellular and molecular adaptations of the heart in response to exercise as well as benefits of exercise in preventing or reversing the pathological remodelling of the fibrotic heart. By furthering our understanding of the beneficial and deleterious roles of cardiac fibroblasts and myofibroblasts and how these roles are related to each other in cardiac development and in heart disease, we may be able to design interventions to prevent the progression of cardiac fibrosis.

Keywords Cardiac fibrosis • Effects of exercise • Molecular pathways

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1 Introduction

Over the last half-century, numerous scientific and clinical reports have examined the relationship between cardiovascular health and physical fitness. The prevailing findings of these studies have concluded that more active individuals tend to develop less cardiovascular disease than their less active counterparts [1]. All types of exercise training have been shown to foster the health and performance of the cardiovascular system. The beneficial effects of exercise can best be explained by the reduction of cardiovascular risk factors as well as the modification of molecular and cellular remodelling pathways in the heart [2]. This review focuses on the beneficial effects of exercise in cardiac fibrosis as demonstrated in basic research studies. Attention will be given to the characterisation of the relationship between exercise and cardiac remodelling, including the molecular and cellular adaptations of the heart in response to exercise as well as benefits of exercise in preventing or reversing the pathological remodelling of the heart [3].

Cardiomyocytes, fibroblasts, and vascular cells in the heart are connected by a complex matrix principally composed of fibrillary collagen. This collagen is instrumental in preserving the structural integrity and plasticity of the cardiac tissue. A cardiac fibroblast is typically described as a cell that synthesizes and secretes proteins that contribute to connective tissue. The heart's matrix, unlike the highly organized connective tissue of bone and tendon, is dense, irregular, and composed of collagens, proteoglycans and glycoproteins. Collagen types I, III, V and VI, as well as fibronectin, periostin and vimentin are some of the structural molecules that are synthesized by cardiac fibroblasts [19]. In the diseased heart, the matrix undergoes structural and subcellular changes that progressively impair cardiac function. Under physiological conditions, fibroblasts secrete extracellular procollagen chains that assemble into cross-linked fibrils in the interstitium. Under pathological conditions, changes in the matrix environment, increased release of cytokines and growth factors, as well as increases in mechanical stress dynamically modulate fibroblast transdifferentiation into myofibroblasts. Higher levels of cross-linking of collagen ensue, leading to increases in myocardial tensile strength [4].

Thus, cardiac fibrosis is characterized by the systolic or diastolic dysfunction that results from the accumulation of extracellular connective tissue proteins in the heart's interstitium. Both clinical evidence and experimental studies have suggested that fibrotic changes in the heart are reversible [5]. Animal models have shown that the beneficial cardiac effects of training are related to signaling pathways involved in hypertrophy and fibrosis. Given our understanding of these signaling pathways, the selection of the animal model that is most appropriate for the proposed research project is of crucial importance to the quality and the eventual translational applications of the research outcomes. From this perspective, this review will focus on providing better insight into the current state of basic research relating to the beneficial effects of exercise in cardiac fibrosis.

2 Cardiac Remodelling and Fibrosis

Cardiomyocytes, fibroblasts, and vascular cells in the heart are connected by an elaborate matrix composed mostly of fibrillary collagen which is instrumental in preserving the plasticity and structural integrity of the heart. Cardiac fibroblasts, which are the most abundant cells in the mammalian heart, have a dynamic but balanced interaction with cardiomyocytes. Historically, the most recognized role of fibroblasts has been their contribution to secretion, maintenance and remodelling of the extracellular matrix. However, fibroblasts have been proposed to participate in many other aspects of myocardial function and dysfunction. For example, the mechanical and electrical contributions of myofibroblasts to the heart before and after injury could be critical [8].

All aspects of these homeostatic interactions are affected in any cardiac injury. Under pathophysiological conditions, the heart's matrix displays significant restructuring and subcellular modification that result in progressively decreased cardiac function. It is now accepted that alterations of the cardiac extracellular matrix and cardiac remodelling play a major role in the development and evolution of cardiac diseases leading to heart failure [6]. Fibrosis is a commonly observed pathological feature of most chronic inflammatory diseases. It normally involves three overlapping inflammatory phases: proliferation, granulation, and maturation. Each of these phases involves the participation of cardiac fibroblasts. The process is characterised by the accumulation of excessive extracellular matrix components, whereby increased synthesis predominates over unchanged or decreased degradation of collagens resulting in excessive, diffuse collagen accumulation in the interstitial and perivascular tissues [4]. Fibrotic remodelling of the heart involves several cell types that participate either directly by producing matrix proteins (fibroblasts), or indirectly by secreting mediators of fibrogenic activity. Part of the secretome that triggers and maintains fibrosis includes myocytes, myofibroblasts, and macrophages/leucocytes/mast cells [4, 11, 12]. This dysregulation of collagen turnover takes place mainly in phenotypically transformed fibroblasts, termed myofibroblasts. In advanced disease, the fibrotic process eventually leads to severe organ dysfunction and death.

In the initial pathophysiology, a significant increase in the release of pro-inflammatory cytokines can be detected from injured cardiac fibroblasts. These cytokines are involved in a feed-forward loop that results in accelerated proliferation, re-expression and upregulation of many of the markers initially expressed within the embryonic and homeostatic stages (see Table 14.1). Eventually, the transformation culminates with the differentiation of fibroblasts into highly proliferative migratory activated myofibroblasts [6, 8].

Myofibroblasts are not only derived from cardiac fibroblasts but can also originate from epithelial cells, endothelial cells, bone marrow-derived cells (fibrocytes), pericytes, and smooth muscle cells [9, 10]. Myofibroblasts have been shown to have important structural, paracrine, and electrical interactions with cardiomyocytes in both development and disease. Acute focal fibrotic scarring follows myocardial

Table 14.1 The relative expression of fibroblast markers in cardiac fibroblast and myofibroblast

Fibroblast marker	Adult cardiac fibroblast	Myofibroblast
Thymus cell antigen-1/CD90	++	++
Vimentin	++	++
Periostin	+/−	++
Discoidin domain receptor 2	+	++
Fibroblast specific protein-1	+/−	+++
α-smooth muscle actin	+/−	+++
Platelet derived growth receptor β	++	++
Fibroblast activation protein	++	++
Stem cells antigen-1	++	++
ADAM metallopeptidase domain 12	+	++
Lysine 6-oxidase	+	+++
Wnt-1-induced secreted protein	++	+++

Adapted from Refs. [8, 19]

infarctions, and due to the limited regenerative capacity of the heart muscle, represents myocardial healing. In contrast, persistent increases in preload or afterload attributable to hypertension, metabolic disorders, valvular disease, ischaemic injury and cardiomyopathies result in chronic diffuse or focal reactive myocardial fibrosis [4, 7]. The dysregulation of distinct pro- and anti- fibrotic factors (i.e. hormones, growth factors, cytokines, chemokines, proteases and reactive oxygen species) is responsible for the alteration of the collagen matrix [13]. The conversion of fibroblasts into active myofibroblasts involves the expression of different cellular markers (see Table 14.1). The conversion of phenotype begins with changes in the subcellular structure such as the onset of expression of α-smooth muscle actin and the increased secretion and assembly of extracellular procollagen chains into collagen type I and type III fibrils that become cross-linked to form the final fibres. The cross-linking of collagen represents a significant post-translational modification as it results in increased myocardial tensile strength and increased resistance of collagen fibres to degradation by matrix metalloproteinases [12, 14]. Fibrosis of cardiac tissue disrupts the myocardial architecture, contributes to myofibrils disarray, and determines mechanical, electrical, and vasomotor dysfunction, thus promoting the progression of cardiac diseases to heart failure [15]. Clinical studies have shown that the severity of histologically-confirmed myocardial fibrosis is associated with higher long-term mortality in patients with cardiac diseases, particularly those with heart failure. From this perspective, detecting, preventing, and reversing myocardial fibrosis have emerged as important novel strategies in the approach to heart failure therapy. Of note, fibrosis persists in the myocardium of heart failure patients under the current treatment regimens recommended by the official guidelines. Thus, the current treatment of heart failure patients, although improving clinical symptoms, does not appear to reverse the underlying fibrosis. In aortic stenosis patients, aortic valve replacements result in regression of left hypertrophy, providing further evidence that hypertrophy and fibrosis appear to be reversible for many cardiovascular diseases [4, 15–18].

3 Molecular Biology of Cardiac Remodelling and Fibrosis

Fibroblasts represent an essential and dynamic cell population in the heart in that they play critical roles in both physiology (development) and pathology (injury). A broad range of molecular signal cascades regulate/modulate the triggering, progression and regression of the fibrotic response. The complexity of the interactions between these signaling cascades greatly complicate the elucidation and understanding of fibrosis at the molecular level, especially given that the relative significance of each pathway varies according to the underlying cause of the fibrotic reaction [25]. The innate and adaptive immune responses of white blood cells and mast cells, along with hormones and growth factors, as well as para/ autocrine (myo)fibroblast and cardiomyocytes signaling all contribute to the intrinsic cellular changes in myocardium. These changes can sustain fibrosis by differentiating, recruiting, activating and stimulating the proliferation of the extracellular matrix–producing myofibroblasts. Differentiating between myofibroblasts and fibroblasts is key to the development of effective therapeutic interventions. Cardiac myofibroblasts were first described in the literature in the 1970s [20]. Distinguishing features of these cells include expanded cytoplasm, microfilament bundles, serrated nuclei and highly defined Golgi complex and endoplasmic reticulum [21, 22]. Recent studies have further contributed by identifying transcription factors associated with various functions of activated cardiac fibroblasts. Two such proteins are sclerosis and myocardin-related transcription factors. Scleraxis plays a role downstream of transforming growth factor beta (TGFβ) and is involved in the synthesis of the extracellular matrix. Myocardin-related transcription factors initiate changes in the cytoskeleton and also upregulate expression of alpha-smooth muscle actin (αSMA) during fibroblast activation [19, 23]. In response to stress signals, the stimulation of gene expression associated with fibrosis requires the intervention of sequence-specific DNA-binding transcription factors. These factors include the transforming growth factor beta signaling proteins SMAD2/ SMAD3, nuclear factor of activated T cells (NFAT), myocardin-related transcription factors (MRTF) and serum response factor (SRF) [29]. To date, the strongest signature discovered in cardiac fibroblasts are the transcription factors the T-box transcription factor Tbx20 and the zinc finger transcription factor Gata4, as opposed to the heterogeneous expression found for the epicardial markers such as Wilms' tumor-1 (Wt1) and epicardin (or transcription factor 21, Tcf21). Other cardiac fibroblasts transcription factors that are shared with both cardiomyocytes and cardiac progenitor cells include members of the T-box family (Tbx2, Tbx5 and Tbx20), members of the GATA family (Gata4, Gata4, Gata6) the muscle marker Myocyte-specific enhancer factor 2C (Mef2c), and the more heart-specific marker Heart and neural crest derivatives-expressed protein-2 (Hand2) [24]. The considerable overlap of gene expression signatures that are shared between cardiac fibroblasts and cardiomyocytes (Table 14.2) are highly suggestive that both cell types could share similar pathways in fibrotic processes and/or that the cardiac fibroblasts could naturally be reprogrammed into cardiomyocytes, were it not for the likely presence of strong endogenous repressors of such transdifferentiation [28].

Table 14.2 Cardiogenic signature of transcription factors expressed in fibroblasts, cardiac progenitor cells and adult cardiomyocytes

Type of cardiac cells	Transcription factors	Overlap in cellular expression
Fibroblasts	Tbx2/5/20; Nkx2–5; Hand2; Gata4/5/6; Mef2c; Wt1; Tbx18	With cardiac progenitor cells and cardiomyocytes
	Tcf21	With cardiac progenitor cells only
	Lbh	With cardiomyocytes only
	Heyl	No overlap
Cardiac progenitor cells	Tbx2/5/20; Nkx2–5; Hand2; Gata4/5/6; Mef2c; Wt1; Tbx18	With fibroblasts and cardiomyocytes
	Tcf21	With fibroblasts only
	Hand1; Mesp1; Isl1; Tead1; Mef2a	With cardiomyocytes only
	Oct4; Nanog	No overlap
Adult cardiomyocytes	Tbx2/5/20; Nkx2–5; Hand2; Gata4/5/6; Mef2c; Wt1; Tbx18	With fibroblasts and cardiac progenitor cells
	Hand1; Mesp1; Isl1; Tead1; Mef2a;	With cardiac progenitor cells only
	Lbh	With fibroblasts only
	Tbx1/3; Srf; Elk1/3/4; Foxh1	No overlap

Adapted from Ref. [24]

Gene expression often requires the participation of specific transcription factors. Transcription factors are a wide variety of proteins that either stimulate or suppress the transcription of genomic DNA into messenger RNA (mRNA). MicroRNAs (miRNA) are small non coding RNA molecules that are of great importance in the regulation of gene expression. The important roles played by miRNAs in the pathological process of cardiac diseases have recently been elucidated and represent one of the most rapidly growing areas of research in cellular and molecular medicine [35, 36]. A growing body of research findings are suggesting that miRNAs regulate many processes observed in the development of cardiac fibrosis such as proliferation, cell death or changes in metabolism, structure, and function. It appears that multiple miRNAs (e.g. the muscle-specific miRNAs miR-1, miR-133 and miR-208, as well as other miRNAs including miR-18b, miR-21 and miR-195) play a role in cardiac fibrosis and that each one of them can determine pathological processes in an independent fashion [36, 37].

On the other hand, recent studies have also clearly suggested a pivotal role for the epigenetic control of gene expression in the pathogenesis of cardiac fibrosis although major knowledge gaps still exist. Epigenetics is a type of gene regulation that results in changes being imparted to DNA or proteins in nucleosomes, without modifications to the underlying sequence of nucleotides. A number of post-translational modification can target histone tails. These modifications can have drastic effects on the expression of genes and are central to the processes of epigenetic control [30–33]. The possibility of epigenetic involvement in the regulation of cardiac fibrosis - through histone deacetylases (HDACs), histone acetyltransferases (HATs), acetyl-

lysine readers, and histone methylation - are reviewed in Stratton and McKinsey (2016) [34]. It is worth noting that, to date, there are no relevant data regarding the effects of exercise in cardiac fibrosis occurring via interactions with epigenetic control mechanisms.

The molecular biology of cardiac remodelling and fibrosis is complicated by the fact that there exists no single signaling pathway specific to cardiac fibroblasts. Furthermore, a better understanding of the communication between cardiac fibroblasts and cardiomyocytes is further complicated by the complex nature of their interactions (not only paracrine signals but structural and electrical signals as well). Inflammatory signals appear to be more important in reparative and ischemic fibroses. These fibroses are associated with significant activation of cytokine and chemokine signaling cascades [26, 27]. The renin-angiotensin II-aldosterone system and fibrogenic growth factors (such as TGF-β and PDGF) appear to be involved in most fibrotic cardiac conditions regardless of aetiology. For a more thorough review of the molecular pathogenesis of cardiac fibrosis, please consult Kong et al. which describes the cellular effectors and molecular pathways contributing to cardiac fibrosis along with a detailed review of the various mediators involved in the fibrotic process: fibrogenic growth factors, matricellular proteins, mast cell-derived proteases, reactive oxygen species, inflammatory cytokines and chemokines, renin-angiotensin II-aldosterone signaling cascade and endothelin-1 [11].

The significant and close relationship that exists between cardiomyocytes and cardiac fibroblasts suggests that it would probably be beneficial to consider both of these cell populations when designing therapeutic interventions that seek to activate regenerative processes. Targeted signals that modulate the activity of myofibroblasts or that seek to activate the survival pathways of cardiomyocytes should be developed in combination to promote better cardiac regeneration and avoid the potential clinical complications of current therapeutic approaches. It is important to underline that both clinical and experimental evidence support the notion that changes resulting from cardiac fibrosis may be reversible.

4 The Beneficial Effects of Exercise in Cardiac Fibrosis

Historically, the treatment of heart disease included rest and strict limitations of physical activity. The past 20 years have seen an almost complete reversal in this way of thinking. It is now commonplace for moderate to vigorous exercise to be highly recommended not only for the prevention but also for the treatment of ischaemic heart disease. Focusing on myocardial fibrosis may potentially improve patient care through the targeted diagnosis and treatment of emerging fibrotic pathways [3]. It is therefore of utmost importance to better comprehend the mechanisms involved in the initial changes, subsequent progression, and eventual resolution of cardiac fibrosis in order to devise the most effective therapeutic approaches. Animal models have allowed researchers to make significant inroads in studying the impact of physical activity on cardiovascular and overall health. In such studies, exercise

has been demonstrated to make important improvements in skeletal muscle function, glucose homeostasis, respiratory muscle strength, locomotor coordination, bone stability and psychological well-being among many others findings. The intensity-controlled treadmill exercise in adult rats produces improved cardiac function and increased myocardial mass through cardiomyocyte hypertrophy as well as new cardiomyocyte and capillary formation. In models of systolic heart failure, endurance training has been shown to promote the reversal of remodeling accompanied by marked improvements in both systolic and diastolic left ventricle functions as well as decreases in diameters measured at the of end of diastole. The reversal of the negative remodeling is attributed to the effects of endurance exercise These exercise-induced improvements include reductions in cardiomyocyte apoptosis and cardiac fibrosis, increases in phosphoinositide 3-kinase (PI3K) activity, improvements in the cardiac handling of calcium, improvements in endothelial function resulting from increases in nitric oxide (NO) production, increases in parasympathetic tone and marked improvements in the antioxidative protection mechanisms of the cardiac muscle [38–40]. Reductions in cardiomyocyte apoptosis can be explained by the activation and subsequent differentiation of cardiac stem cells and progenitors [43]. The signaling cascade most often characterized as mediating physiological cardiac growth is the insulin-like growth factor-1 (IGF-1)-PI3K(p110α)-Akt pathway. The activity of this pathway and ensuing downstream phosphorylation of Akt substrates were increased in the hypertrophic hearts of transgenic mice overexpressing the IGF-1 receptor (IGF-1R). Akt, (also known as protein kinase B), is a well-characterized serine/threonine kinase that is targeted by PI3K. Of the three Akt isoforms, Akt1 and Akt2 are expressed at high levels in cardiac tissue [45–47]. Moreover, in mice that had undergone endurance swim training, cardiomyocyte hypertrophy and renewal were observed. These beneficial changes were dependent on decreased expression of the transcription factor CCAAT/enhancer-binding protein beta (CEBPβ). Interestingly, expression of Akt that was specifically targeted to the nucleus of cardiac tissue of transgenic mice resulted in prolonged cycling of postnatal cardiomyocytes and expansion of the c-kit^{pos}-Nkx-2.5^{pos} cardiac progenitor cell population [44].

Clearly, animal models have provided researchers with solid evidence that supports a direct link between the beneficial effects of training and intracellular signaling pathways responsible for hypertrophy and fibrosis in the heart. At the molecular level, recent studies in animals have suggested that activation of the PI3K (p110) pathway could be implicated in exercise-induced cardioprotection. In one study, levels of interstitial fibrosis were significantly reduced thereby improving survival by approximately 20% [41]. In a rat model of ischemic heart failure, exercise training resulted in a marked decrease in the expression of angiotensin-converting enzyme mRNA as well as angiotensin II-1 receptors (AT1) in myocardial tissue after a 2 month training regimen with a treadmill. Given that almost all of the angiotensin II found in the heart (>90%) is produced locally within the cardiac muscle, this particularly important finding implies that angiotensin II levels are significantly reduced locally, in the heart, as a consequence of exercise training. This local decrease in angiotensin II activity also results in decreased fibrosis. The evidence

supporting their conclusion of reduced fibrogenesis included decreased expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) with unchanged expression of matrix metalloproteinase-1 (MMP-1) as well as reduction of the collagen volume fraction in the exercised animals [42].

One of the most significant advancements in the study of gene expression regulation has been the recent elucidation of the important roles of miRNAs. A compilation of studies has concluded that a single miRNA can target hundreds of different mRNA species, each with a varying degree of efficacy. Since an individual mRNA can be affected by many different miRNAs, one can only imagine the very elaborate and complex nature of the regulatory control systems that miRNA could impose on gene expression programs. A number of miRNAs have also been shown to modulate intracellular events such as hypertrophy, muscle recovery, the metabolism of mitochondria as well as inflammatory processes. They are therefore an interesting and relevant way of evaluating the body's response to physical exercise. The characterization of patterns of miRNA expression that are most associated with the effects of exercise and training could prove useful in the estimation of physical performance capacity and the tracking of muscle fatigue and recovery [52]. Two miRNAs, miR-1 and miR-133, were found to be decreased in two models of physiological cardiac hypertrophy. One model used transgenic mice with the selective cardiac overexpression of a constitutively-active Akt kinase and the other model displayed cardiac hypertrophy that was induced in exercised trained rats [48, 49]. In rats undergoing a training program of aerobic swimming, the expression of miR-29c was increased. Furthermore, downregulation of miR-29 increased the accumulation of collagen and worsened fibrosis in the heart whereas the overexpression of miR-29 resulted in the opposite effects [50, 51]. Some newly described microRNA molecules such as miR-17-3p might serve as a novel therapy in association with exercise for enhancing cardiac survival and regeneration [53].

5 Conclusion

Fibroblasts are essential and dynamic cells in the mammalian heart. They are crucial to cardiac development and to the response to injury. Fibroblasts establish and maintain the mechanical, biochemical, and electrical environment of the heart through their intricate interactions with cardiomyocytes. Cardiac injury disrupts the balance between fibroblasts and cardiomyocytes and creates a state favouring inflammation and fibrosis. This adaptive response initially serves to increase wound healing. If homeostasis is not regained, however, the heart may be damaged and heart failure may ensue.

Myofibroblasts are mediators of both the adaptive and maladaptive components of this reaction. By furthering our understanding of the beneficial and deleterious roles of cardiac fibroblasts and myofibroblasts and how these roles are related to each other in cardiac development and in heart disease, we may be able to design interventions to prevent the progression of cardiac fibrosis.

Exercise is a potent stimulator that activates numerous downstream cascades at the molecular and cellular levels. When exercise is sufficiently intensive and sustained, it results in cardiac remodeling. This remodeling increases cardiac functional capacity in both healthy and diseased individuals.

Aerobic exercise capacity is a prognostic marker of heart disease. Clinicians should promote the benefits of exercise for patients with all levels of cardiac fitness including those who exercise casually, sedentary individuals and patients with established cardiovascular disease.

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Chapter 15
Physical Exercise Is a Potential "Medicine" for Atherosclerosis

Jian Yang, Richard Y. Cao, Rongrong Gao, Qiongyao Mi, Qiying Dai, and Fu Zhu

Abstract Cardiovascular disease (CVD) has been recognized as the number one killer for decades. The most well-known risk factor is atherosclerosis. Unlike the acuity of CVD, atherosclerosis is a chronic, progressive pathological change. This process involves inflammatory response, oxidative reaction, macrophage activity, and different interaction of inflammatory factors. Physical exercise has long been known as good for health in general. In recent studies, physical exercise has been demonstrated to be a therapeutic tool for atherosclerosis. However, its therapeutic effect has dosage-dependent effect. Un-proper over exercise might also cause damage to the heart. Here we summarize the mechanism of Physical exercise's beneficial effects and its potential clinical use.

Keywords Exercise • Cardiovascular disease • Atherosclerosis

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1 Introduction

Cardiovascular disease (CVD) remains the leading cause of death in the world. The dramatic clinical events, such as unstable angina (UA), myocardial infarction (MI) and stroke are all caused by atherosclerotic process. Atherosclerosis can start to form as early as childhood, and progress to adulthood [1]. Over the last several decades, people have become more obese and less physically active. Thus, the incidence of disease related to metabolic dysfunction, such as diabetes, hypertension and hyperlipidemuia, has been increased dramatically. In addition, according to the WHO database, about 80% of CVD related mortalities are caused by unhealthy behaviors, such as high fat diets, physical inactivity, alcohol abuse and so on. It has been accepted that changing lifestyle could reduce the incidence of these disease. One of the most cost-effective intervention is physical exercise [2, 3]. Recent studies have revealed that physical exercise decreases CVD related morbidity and mortality through systemic and cardiac-specific adaptations. Furthermore, physical exercise has been proven to be a very promising tool in both primary and secondary prevention of CVD [4–6].

Atherosclerosis has been considered as a chronic inflammatory artery disease, which is responsible for approximate 50% of deaths worldwide. Risk factors to atherosclerotic diseases include smoking, diabetes, hypertension, hyperlipidemia and lack of physical activity [7]. This process is initiated by circulating plasma low-density lipoprotein (LDL) entering the sub-endothelial space in the blood vessel. In artery with normal endothelial function, LDL would be cleared. However, if endothelial malfunction exists, the balance of entering and clearance would be broken and LDL would keep accumulating. With time goes by, the accumulated LDL would build up plaque inside the arterial wall, which could result in narrowing of the lumen. Consequently, the narrowed lumen reduces blood supply to the end organs. In some circumstances, the plaque could become vulnerable and finally ruptures. The ruptured plaque could lead to thrombus formation, which critically obstructs the blood flow.

To date, it has been well established that atherosclerosis is the result of interactions of increased oxidative stress, inflammation, macrophage dysfunction, endothelial injury, lipid deposition, and genetic predisposition [8, 9]. Physical inactivity has been widely believed to be an independent risk factor for atherosclerosis and cardiovascular complications [10]. It contributes to the accumulation of visceral fat and the activation of inflammatory pathways that promote the development of metabolic disorders [11–13]. Nevertheless, evidence suggested that physical exercise was able to reverse this pathological changes [14, 15].

2 Physical Activity and Atherosclerosis

Atherosclerosis is a complicated process involving various reactions. It is started from LDL entering the sub-endothelial space in the blood vessel, which is later oxidized by reactive oxygen species (ROS). Oxidized LDL upregulates adhesion molecules and induces the expression of chemotactic agents in endothelial cells

[16]. These factors are able to recruit inflammatory cells to the vessel wall, which in turn can induce cascade expression of inflammatory factors, such as MCP-1, and cytokines including interferon Gamma (IFN-y), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [17, 18]. Later on, smooth muscle cells (SMC) migrate from the tunica media into the intimal or sub-endothelial space and participate in the reaction. Finally, a fibrous cap would be built [19].

Recent studies demonstrated that physical inactivity can lead to the accumulation of visceral fat and consequently result in the activation of oxidative stress and inflammation cascade, which eventually enhances the progression of atherosclerosis [20]. Regular physical exercise confers plentiful effects in restraining the atherogenic process, which involved arterial wall remodeling, plaque size modulation, macrophage function regulation and inflammatory reaction control [21]. In animal study, regular physical exercise corrects cardiovascular and metabolic risk factors to baseline level in obese rats that were fed on a high-fat diet [22]. On the other hand, exercise can prevent the conversion of plaques into a vulnerable phenotype [23], which is the main trigger of acute coronary syndrome. Randomized clinical trials have validated the role of physical exercise in primary and secondary prevention of atherosclerosis, CVD, and decrease mortality among adults [24, 25].

2.1 Physical Exercise Reduces Atherosclerosis Process

Physical exercise prevents atherosclerotic plaque development and induces the regression of coronary stenosis possibly by preventing and reducing inflammatory reaction, oxidative stress and regulating endothelial function. Besides, physical exercise can normalize blood pressure, insulin resistance, serum lipid level [15], which all are crucial factors during atherosclerosis development.

2.2 Physical Exercise Exerts Anti-inflammatory Effects

Chronic inflammation is one of the most important features of atherosclerosis and persists throughout the whole process. It starts with the release of pro-inflammatory factors including cytokines and nuclear factor-κB (NF-κB). NF-κB, a pro-inflammatory transcription factor, can upregulate the transcription of other pro-inflammatory molecules, such as tumor necrosis factor-α (TNF-α), interleukins (IL-1β, and IL-6), cyclo-oxygenase-2 (COX-2), and nitric oxide synthase (iNOS). Apart from this, it is also associated with oxidative stress production and disease related to aging [26–28]. One study showed that combination of exercise and Korean red ginseng supplement or exercise alone could decrease serum CRP, NF-kB, TNF-α, COX-2, IL-6, ICAM-1 and VCAM-1 in aorta of D-gal induced aging atherosclerotic rats [29]. Another report showed that higher intensity exercise enhanced NF-κB activation, which consequently bring adverse effect to CVD [30, 31].

Being able to regulate the activity of cytokines may contribute to the protective effect of physical exercise [32, 33]. TNF-α levels are found to be increased in patients with atherosclerosis [34–36]. It activates and accelerates atherogenesis process by promoting thrombosis, vascular remodeling, inflammation, endothelium apoptosis, oxidative stress and impairing NO bioavailability [35, 37, 38]. Apart from that, TNF-α precipitates in the secretion of adhesive molecules, thus encouraging recruitment of inflammatory cells [36]. Overexpression of TNF-α is implicated in damaged arterial wall and unstable plaque [39]. The reduction of TNF-α could be achieved by lipopolysaccharide-stimulated monocytes in whole blood from healthy subjects [40]. Further study looking into physical exercise and TNF-α found that physical exercise could prevent the elevation of circulating TNF-α [41]. Other studies also showed that physical exercise decreases cytokines, in particular TNF-α [42–44], which is a crucial risk actor in atherosclerosis development and vascular function. TNF-α plays a central role in vascular inflammation, involved in oxidative stress, apoptosis and also thrombogenesis [45–47].

IL-6 has received increasing attention as it interprets the anti-inflammatory effect of physical exercise in patients with CVD [48, 49]. In contrast to TNF-α, IL-6 inhibits the endotoxin-induced increase of TNF-α [41], induces concentrations of two other anti-inflammatory cytokines: IL-1Ra (IL-1 receptor antagonist) and IL-10, and has a central role in exercise-induced leukocyte trafficking [50]. Furthermore, IL-6 has vital effect on atherosclerosis by producing CRP. CRP can increase the levels of reactive oxygen species (ROS) and NF-κB. Both of them can precipitate inflammation [32, 51]. Also, CRP is associated with higher cardiovascular risks [32, 52]. A recent review has summarized that physical exercise could lower the effects of CRP on inflammation of atherosclerosis [53]. Increasing evidence has clarified that physical activity ameliorated activation of inflammation, by decreasing level of TNF-α, IL-1β, and IL-6. In addition, it activates matrix metalloproteinase 9, thus in turn attenuates fibrosis, in an MI animal model [50, 54].

IL-18 is another pleiotropic inflammatory cytokine, which was found to be increased in the serum of type II diabetes patients. It revealed itself a predictor of cardiovascular death and future CVD [55]. Besides, it worsens the plaque burden and is related to left ventricular myocardial dysfunction [56]. IL-18 can be reduced by an exercise intervention without weight change [57, 58].

In summary, physical exercise is an effective way to decrease the key inflammatory factors like TNF-α, CRP, IL-6 and IL-18, suppressing atherosclerosis from a molecular level.

2.3 Physical Exercise Exerts Antioxidant Effects

Oxidative stress is another important pathology change in atherosclerosis. It has been well established that physical exercise has a strong negative effect on oxidative stress [59–62]. Oxidative stress is defined as an imbalance between the excessive production of oxidant compounds plus the insufficient anti-oxidant defense

systems, which may cause tissue injury. It can produce endothelial dysfunction and accelerate atherosclerosis in patients with CVD.

Oxidized LDL (OxLDL), formed under oxidative stimulation, can enhance local inflammatory response. Lots of factors contributing to the formation of OxLDL. Systemic diseases like diabetes, chronic kidney disease are well known to induce OxLDL [63]. Air pollution, which is usually neglected, is also a strong inducer for systemic oxidative stress [64]. Physical exercise has been proven to ameliorate systemic inflammation and oxidative burden via acting on NO [65, 66].

It has been well studied that NO is involved in the oxidation of LDL-cholesterol [67, 68]. Decreased endothelial NO bioavailability may be the earliest indication of atherosclerosis [69]. Reduction of endothelial NO bioavailability is closely related to vasoconstriction, platelet adherence and aggregation, leukocytes adherence, and increased proliferation of SMC [70]. These effects all contribute to the pathogenesis of atherosclerosis. Decreased expression of endothelial NO synthase (eNOS), loss of eNOS activity, and accelerated NO degradation by ROS are associated with suppressed NO bioactivity [71, 72]. Exercise readjusts the balance between NO generation and NO inactivation [73]. Among many enzymatic systems that are able to produce ROS, NADPH oxidase appears to be the most significant one [74, 75]. Physical inactivity increases the activity of NADPH oxidase, followed by enhanced O2− and ROS production. It finally will lead to endothelial dysfunction and atherosclerotic lesion progression [76]. To sum up, the mechanism of exercise modulating oxidative stress are as follows: (1) increases eNOS expression and/or eNOS Ser1177 phosphorylation [mediated by an increase in Akt expression and/or phosphorylation]; (2) increases antioxidant superoxide dismutase (SOD) expression; (3) decreases NADPH oxidase activity and expression of its subunits (gp91phox, p22phox and nox4), leading to reduced ROS generation [77–82].

Last but not the least, hyperhomocysteinemia(HHcy) is an unneglectable risk factor for atherosclerosis and oxidative stress. HHcy also involved in vascular responses and endothelial injury [83]. It could enhance propensity for plaque rupture and promote vascular SMCs proliferation [84–87]. Studies have elucidated that HHcy induces oxidative stress/ROS through induction of thrombin and activation of PAR-4 and NADPH oxidase 1, or oxidation of reactive sulfhydryl groups in the presence of molecular oxygen [83, 88, 89]. Exercise is found to be effective in suppressing HHcy induced destruction. Firstly, exercise can reduce HHcy-mediated oxidative stress and atherogenesis, either directly by reducing Hcy levels or indirectly by enhancing PON1 levels. PON1 is a calcium-dependent esterase belonging to the PON family of proteins and is strongly associated with HDL level. PON1 can reduce cellular oxidative stress as well as the rate of cholesterol biosynthesis after entry into the macrophages [90–92]. Secondly, exercise can upregulate kidney betaine homocysteine S-methyltransferase level, which removes Hcy through the nonclassical remethylation pathway [93]. In turn, HHcy can also restrict the physical activity capacity. Therefore, it is inevitable to correct the HHcy before the exercise regimen to exert its full potential [94–96].

Physical Exercise Regulates Endothelial Function

Endothelial cell integrity is crucial for preserving vascular homeostasis. It allows the continuous adjustment of vascular tone, regulation of leukocyte traffic, and also maintenance of blood fluidity [97]. Endothelial dysfunction refers to an injury of endothelium-dependent vasorelaxation. Vulnerable plaques are sites of active inflammation and oxidative stress. They are most likely to locate where there is impaired endothelial function. Endothelial dysfunction presents in all stages of atherosclerosis process. The impaired endothelial cells release lower levels of NO, thrombomodulin, prostacyclin and tissue plasminogen activator but increase the release of endothelin-1, angiotensin II, plasminogen activator inhibitor (PAI)-1 [98, 99]. However, clinical and experimental findings clearly demonstrate that physical exercise can counteract these destructive effects [100, 101]. Researchers found that a primary target of the physical exercise intervention appears to be the impaired endothelial function [102, 103].

Inflammatory factors like NO and CRP are crucial in endothelial homeostasis. Loss of NO bioactivity seems as an early event in the pathogenesis of atherosclerosis [69]. CRP is produced in response to IL-6, and its pro-atherogenic effects are applied through damaging the endothelial function. Both of them could be downregulated by physical exercise.

Interestingly, researchers also found that endothelial impairment is accompanied by increased blood pressure, insulin resistance and dyslipidemia [104, 105]. This suggests that the concurrent appearance of these risk factors might share a common mechanism. Given this information, physical exercise preserves endothelial function by controlling blood pressure through regulation of AII receptor (type I) and increasing skeletal muscle endothelial nitric oxide synthase content [106, 107]. By controlling one factor, physical exercise helps to decrease the risk for all other chronic metabolic disease. Besides, another study demonstrated that acute dynamic resistance exercise can decrease resting blood pressure and reactivity to phenylephrine and increased endothelium-dependent relaxation [108].

In patients with CVD, physical exercise reverses endothelial dysfunction and increases CBF [109–111]. While in patients with Type 2 diabetes and obesity, similar results have been observed [112, 113] but without concomitant changes in traditional risk factors. All these results encourage physical exercise for both treatment and prevention of these endothelial function-centered disease [114–117].

2.4 Physical Exercise Reduces Endothelial Adhesiveness

Endothelial adhesiveness plays important role in the development of atherosclerosis. Within a week after the initiation of a high-cholesterol diet, monocytes adherence to the endothelium and starts to migrate. It leads to the development of intimal lesions, which contain sub-endothelial macrophage-derived foam cells, small numbers of non-lipid-filled macrophages and T lymphocytes [118, 119]. Under normal physiologic conditions, endothelial cell does not secrete factors that induce the adhesion molecules. Once activated by cytokines, oxLDL, or ROS, endothelial cells

will start to express cell adhesion molecules (CAMs), such as ICAM-1, VCAM-1, E-selectin, and P-selectin. They are all essential to the recruitment of inflammatory cells [120]. While physical exercise has a positive effect in the circulating CAMs. Circulating ICAM-1, VCAM-1, and P-selectin are found to be significantly decreased after 2 weeks of exercise training [121–123]. Similarly, physical exercise performed 5 times per week for 6–8 weeks decreases circulating P-selectin andV-CAM-1 levels [124]. Shear stress induced during physical exercise could probably contribute to these effects [125]. Besides, apart from the direct impact on CAMs expression, physical exercise might also have indirect beneficial effects throughout the reduction of agonists of CAM synthesis [43, 126, 127].

Endothelin-1 (ET-1), which is expressed by vascular endothelial cells, has strong constrictor and proliferative activity on SMCs. Because of this, it has been implicated in modulation of vascular contraction and progression of atherosclerosis. Its production has been found to be elevated in human atherosclerotic lesions [128–130]. It was found that in healthy adults, physical exercise is able to suppress its level [131, 132].

In all, by reducing the soluble adhesion molecules which may represent the interaction between activated monocytes/macrophages and endothelial cells and ET-1 concentration, physical exercise might be considered as an effective non-pharmacological intervention to reduce endothelial adhesiveness.

2.5 Physical Exercise Regulates Macrophages Function

Macrophage has been studied for centuries for its role in inflammatory response. In addition to modulating immune reaction, it is also noticed to take great part in the atherosclerotic process. Macrophage is able to modulate lipid metabolism. During the early phase of plaque formation, macrophages become foam cells when it can not process OxLDL. Foam cells are the hallmarks of atherosclerotic lesions and vulnerability [133, 134]. Macrophages have been parsed into at least two subtypes(M1 and M2), each of which have specific roles in atherosclerosis [135–138]. M1 macrophages produce high levels of pro-inflammatory factors like CD40, CD80, IL-6, TNF-α, iNOS [139]. While M2 macrophages leads to more foam cell formation with higher phagocytic nature and greater ability to import OxLDL. Foam cell–prone M2 macrophages level is higher than pro-inflammatory M1 macrophages in the early atherosclerotic lesions; but the ratio reverses as the lesion progresses [140]. In addition, macrophages express MMPs to stable plaques during atherogenesis [141–143]. Additionally, HHcy may be a primary cause for the macrophages dysfunction that leads to the effect on atherosclerosis [142, 144].

Physical exercise prevents foam cell formation. It accelerates the transportation of cholesterol from macrophages to the liver which has been considered as the initial step of atherosclerosis [145]. Moreover, physical exercise encourages accumulation of collagen and elastin by modulating serum level of MMPs and TIMP-1. This greatly keep the plaque stability and reduce in lesion incidence and arterial stenosis [146–148].

2.6 *Physical Exercise Preserves Atherosclerotic Plaque Stability*

Most of the acute coronary syndrome (ACS) occurs because of plaque rupture. Plaque rupture exposes sub-endothelial to various of thrombogenic factors in the blood. Thrombus forms immediately after the exposure, leading to acute myocardial infarction or stroke [16, 18]. The vulnerability renders plaques to rupture. A vulnerable atherosclerotic plaque has thin fibrous cap, large lipid core (>50% total plaque surface), high inflammatory cell burden but low volume of SMCs [149].

Physical exercise has shown its capacity to slow the progression of atherosclerosis via promoting plaque stability and preventing plaque rupture in animal studies [76, 106, 150, 151]. ApoE −/− mouse model has been commonly used in atherosclerosis studies. Using this model, swimming training was applied to study its effect on the plaque. After swimming training, it is observed that the plaques are more stable by thicker fibrous cap, less adventitia inflammation, decreased media degeneration and inflammatory macrophage plaque content [106]. Physical exercise regulates plaque size and its rupturing potential through regulation of matrix content and matrix regulators [151]. Physical exercise decreases MMP-2, MMP-3, and MMP-8 levels as well as IL-6. Besides, collagen, elastin, and TIMP-2 (inhibitor of MMP-2 and MMP-9) were also found to be increased in parallel with the change of fibrous cap thickness [147, 151]. In other studies, MMPs, TIMP-1 are also found to be modulated by physical exercise [152].

Apart from the ability to modifying lipoproteins, macrophages can worsen the plaque status by producing MMPs, which is known to degrade collagen in plaque [153]. Collagen is the basic structure to keep the atherosclerotic plaque well-formed. Destruction of the collagen would lead to thrombogenic disasters as mentioned above. Physical exercise could prevent this from happening by modifying macrophage function.

Pro-inflammatory enzyme lipoprotein-associated phospholipase A2 (Lp-PLA2) is a novel marker for plaque inflammation and rupture-prone plaques [154]. Lp-PLA2 binds to ApoB-containing lipoproteins and degrades oxidized phospholipids in LDL-cholesterol. Elevated level of Lp-PLA2 can be detected within the necrotic core and macrophages of vulnerable plaques, but not in early stable plaques [154]. Furthermore, Lp-PLA2 predicts mortality in MI and post-MI patients [155]. Physical exercise has negative effects on this new biomarker, however, clinical evidence remains insufficient [156, 157]. In one clinical study, patients with dyslipidemia have suppressed level of Lp-PLA2 after strict lifestyle modification [158].

High plasma Hcy concentration is associated with atherosclerotic plaque rupture and morbidity in type 2 diabetes patients, and is considered as an independent risk factor for CVD [84–86, 159–161]. It encourages plaque maturation by activating SMCs and promoting macrophage differentiation. While physical exercise can potentially reduce the detrimental effects of HHcy on macrophages. It should be noted that this effect has not be tested in vivo so far.

3 Physical Exercise, Can One Overdose?

With all the evidence above, the agreement that physical exercise can be applied to treat atherosclerosis can be reached. Like regular medicine, physical exercise also has dosage effect. Different dosage of physical exercise refers to the time and strength spent on exercise. Regular intense exercise brings cardiac adaptations which comprises the clinical constellation of findings known as the athlete's heart [162–165]. However, increasing studied found that these adaptations may also have deleterious effects. For example, some reports have claimed that atherosclerotic plaques are present in the carotid or peripheral arteries of 90% of marathon runners at the age of 50–75 [166, 167].

Regular and moderate-intensity exercise, on the other hand, reduces cardiovascular morbidity and mortality. It could be served as primary and secondary prevention of CVD [165, 168–171]. Vigorous exercise training will cause sport-specific hemodynamic alterations, leading to profitable structural and functional adaptations in athletes [172–174]. Chronic exposure to high levels of exercise training, which is equivalent to "exercise overdose", may bring some adverse effects. Long term stress on the heart will cause cardiac remodeling. The clinical presentation could be atrial fibrillation and cardiomyopathy [175, 176]. "Overdosed" exercise does more harm than good. To testify this, 40 elite endurance athletes were included in one study. A decreased right ventricular systolic function and increased cardiac injury biomarkers were detected right after completion of an ultra-endurance exercise. Although in this study short-term recovery appears complete, chronic structural changes and reduced RV function have been observed in some athletes [176, 177].

4 Summary

In summary, regular physical exercise is highly beneficial in reducing the risk of atherosclerosis development, and the underlying mechanism could be concluded as followings: (1) reducing of pro-inflammatory cytokines; (2) counteracting oxidative stress via decreasing ROS production, Hcy level, NADPH oxidase activity and increasing NO availability; (3) improving endothelial function; (4) decreasing endothelial adhesiveness by modulating the expression of ICAM-1, VCAM-1, E-selectin, P-selectin and ET-1; (5) regulating macrophage function and suppressing the foam cell formation; (6) lowering LDL and triglyceride levels. (7) preserving atherosclerotic plaque stability. Similar to medicine, the beneficial effect of PE has dosage effect. Overdosing would also bring "toxicity". Vigorous exercise training could adversely affect cardiac function and ameliorate all these beneficial effects. More clinical trials regarding to the proper exercise training are needed to establish a more mature physical exercising treatment system.

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Chapter 16
Experimental Evidences Supporting Training-Induced Benefits in Spontaneously Hypertensive Rats

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Abstract It is well known that chronic hypertension is accompanied by several functional deficits in the central nervous system and peripheral tissues, most of which are corrected by exercise training. However, the biological mechanisms underlying these effects are not yet well understood. In the present chapter we summarize recent experimental evidence on cellular/molecular mechanisms supporting not only the deleterious effects of hypertension on autonomic control and peripheral circulatory deficits, but also their reversion by low to moderate aerobic exercise training. Interestingly, both hypertension and aerobic training exert their effects by acting exactly on the same pathways/mechanisms but in opposed directions.

Keywords Training-induced • Spontaneously hypertensive rats • Exercise

1 Introduction

The development of experimental models of hypertension allowed researchers to reveal several pathophysiological mechanisms and to discover new therapeutic strategies. Several pharmacological as well as life style modifications have been extensively used to overcome many of the deleterious effects caused by the maintenance of elevated pressure levels. Hypertensive animals submitted to aerobic exercise training, an important life style change, developed numerous cardiovascular

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benefits opening the possibility for its usage in the clinical practice. For instance, clinical studies demonstrated that central autonomic adaptations induced by aerobic training are the main cause of baroreflex sensitivity improvement in hypertensive patients [1]. Indeed, experimental studies in spontaneously hypertensive rats (SHR) identified that normalization of baroreflex function and improvement of cardiac vagal activity are timely correlated with both the downregulation of brain renin-angiotensin system and the reduction of oxidative stress and inflammatory profile in autonomic control areas [2, 3]. The present chapter, reviewing these data and others focused in the cross-talking between tissue dysfunction and molecular/cellular responses, allows the physical training prescribers to understand the physiological mechanisms that attenuate autonomic dysfunction and how the improvement of autonomic regulation contributes to a better circulatory control in hypertension.

2 Central Nervous System and Autonomic Dysfunction in Hypertension

Directly coupled to cardiovascular system, central nervous system drives both acute and chronic hemodynamic adjustments during distinct environmental conditions. For this purpose, the brain continuously monitors the cardiovascular parameters and integrates these signals in order to reflexly codify cardiovascular and metabolic parameters through the sympathetic and parasympathetic divisions of the autonomic nervous system. There are three major sets for afferent signaling of cardiovascular parameters: arterial baroreceptors, peripheral chemoreceptors and cardiopulmonary receptors. These intrinsic receptors of the cardiovascular system codify pressure levels, circulating blood gases and cardiac function, respectively, whose signals are integrated in central autonomic areas, triggering appropriate parasympathetic and sympathetic outflow to heart and vessels [4, 5]. In hypertension, peripheral signaling mainly by baroreceptors and chemoreceptors are dysfunctional and central integrative autonomic mechanisms are abnormal, contributing to increased sympathetic nerve activity and suppressed parasympathetic nerve activity, which characterizes the concept of autonomic dysfunction [6–8].

Baroreflex is recognized as the most important beat-to-beat regulatory mechanism of arterial pressure. Baroreceptors are located in the adventitial and media tunica of aortic arch and carotid sinus. These mechanoreceptors present channels of Degenerin/Epithelial Na + channel, Acid sensing ion channel 2 [9], and transient receptor potential cations channels superfamilies, as the transient receptor potential channel 5 [10]. Once the pressure wave strains the vascular wall in the aortic arch and carotid sinus, the baroreceptors are stretched and the mechanosensitive channels induce a cationic influx, which depolarizes Na + channels and increases aortic depressor nerve activity. In the brainstem, second order neurons located at nucleus tractus solitarii (NTS) are stimulated, activating parasympathetic areas, as the nucleus ambiguus (NA) and the dorsal nucleus of vagus nerve (DMV) [4]. Also, these

glutamatergic second order neurons activate caudal ventrolateral medulla's (CVLM) neurons, which are gabaergic and inhibit the sympathetic premotor neurons within the rostral ventrolateral medulla (RVLM). These neuronal connections activate parasympathetic preganglionic neurons and restrain sympathetic outflow to produce acute cardiovascular adjustments as cardiac output reduction, peripheral vasodilation and decrease in circulating catecholamines, renin and vasopressin, which contribute to reduce and stabilize arterial pressure. On the other hand, during blood pressure reduction, baroreceptors' depolarization is attenuated and, consequently, NTS stimulation. Then, parasympathetic pre-ganglionic neurons of the NA and DMV are not activated, as well as the sympathetic premotor neurons of the RVLM (which received continuous stimulation from higher integrative centers) are not inhibited. Therefore, the arterial pressure fall reflexly leads to unload of parasympathetic and increase of sympathetic activity to bring pressure back to control levels. It is also known that brainstem integrative autonomic areas are continuously modulated by hypothalamic neuronal circuitries within the paraventricular nucleus (PVN) and other supramedullary pathways [5, 7, 11]. Vasopressinergic and oxytocinergic projections from the PVN to the NTS/DMV area are shown to decrease and increase baroreflex sensitivity, respectively [12–16].

Baroreflex sensitivity is attenuated in both pre-hypertensive and hypertensive animals [17, 18]. In other words, the magnitude of cardiovascular adjustments, as changes in heart rate and peripheral vascular resistance, evoked by arterial pressure oscillations are depressed. Stiffening of the arterial wall, oxidative stress and inflammation in autonomic areas are the main mechanisms that generate baroreflex dysfunction [6, 8, 19, 20]. As described in the following sections, hypertensive subjects exhibit several morphological alterations in the wall of arteries resulting in a stiff vascular wall. As a direct consequence, each pressure wave reduces its mechanical deformation leading to an attenuated activation of NTS' second order neurons, reduced reflex responses to load/unload of baroreceptors and elevated pressure variability [21, 22]. The increased pressure variability augments hydrostatic pressure oscillations in the capillaries, exposing tissues to short periods of hypoxia and hyperoxia. These repetitive ischemia-reperfusion episodes activate local renin-angiotensin system, increase reactive oxygen species availability and pro-inflammatory cytokines production facilitating the development of end-organ injuries in several tissues [20, 23–26]. In addition to increased pressure variability, autonomic dysfunction promotes end-organ damage through elevated adrenergic signaling. Beta-adrenergic signaling in the myocardium induces cardiac hypertrophy, augments matrix metalloproteinase-2 activity and enhances TGF-β expression and collagen I and III synthesis. Increased cardiac sympathetic signaling has been shown to intensify reactive oxygen species production and infiltration of hematopoietic mononuclear cells [27–29]. Adrenergic hyperactivation also modifies renin secretion and sodium/water reabsorption determining abnormal renal function [30]. Indirectly, renal adrenergic signaling elevates renin release and, consequently, increases plasma angiotensin II, which, as described subsequently, promotes several tissue injuries through activated oxidative stress and inflammation.

Peripheral chemoreflex, one of the most important regulatory mechanisms for respiratory responses [31], is also crucial for the regulation of autonomic control. Chemoreceptors are located bilaterally in the carotid body, at the bifurcation of common carotid artery. Within the carotid body chemosensitive type I cells are responsible for the transduction of PO2 (most important), PCO2 and pH levels in action potentials' frequency. Acute reduction of PO2 increases AMP/ATP ratio leading to AMP-activated protein kinase (AMPK) activation, which inhibits oxygen-sensitivity potassium channels inducing the chemoreceptor depolarization [32]. The hypoxic environment also attenuates nitric oxide and carbon monoxide production and intensifies reactive oxygen species formation, which promotes further type 1 cell depolarization [33, 34]. From the carotid body's cells, afferents fibers stimulate chemosensitive second order neurons located in the NTS, which project and activate rostral ventrolateral medulla sympathetic pre-motor neurons [35] producing reflex cardiovascular adjustment to hypoxia, as the elevation of peripheral vascular resistance, cardiac contractility, cardiac output and arterial pressure.

Chemoreflex dysfunction in hypertension was described since early 80's in SHRs [36]. In the last years, the role of chronic chemoreceptor activation in the establishment and maintenance of autonomic dysfunction and elevated arterial pressure was demonstrated in experimental studies. Juvenile SHRs in the pre-hypertensive phase already exhibit hyperactivity of carotid body cells [37–39]. Interesting, selective denervation of the carotid bodies in SHRs decreases both sympathetic activity and arterial pressure [37, 40].

2.1 Brain Cellular/Molecular Mechanisms Generating Autonomic Dysfunction

Besides the previously mentioned vascular abnormalities, hyperactivity of the renin-angiotensin system, increased availability of reactive oxygen species and pro-inflammatory cytokines were described in the brain of hypertensive individuals. In angiotensin II-induced hypertension, angiotensinergic and inflammatory signaling are shown to increase NADPH oxidase activation, superoxide anion release and neuronal calcium influx in circumventricular areas as the subfornical organ. Since the subfornical neurons project and activate sympathetic pre-motor neurons located in the PVN, increased neuronal activity in the subfornical organ leads to baroreflex dysfunction, increased sympathetic outflow and elevated blood pressure [41–45]. In several models of hypertension, such as the SHR [2, 3, 46], angiotensin II-induced [44, 47–51], renovascular [52–54] and high salt diet hypertension [55], in addition to areas outside the blood-brain barrier, angiotensinergic, oxidative and inflammatory signaling were also observed in autonomic brain areas inside the blood brain barrier as the PVN, RVLM and NTS.

There are many cellular/molecular mechanisms by which RAS hyperactivity and oxidative stress promote neural inflammation and autonomic unbalance. Increased gene and protein expression of different components of the vasoconstrictor axis of the renin-angiotensin system as the angiotensinogen, angiotensin converting enzyme and AT1 receptor were described in the brain of hypertensive animals [49, 53, 56, 57]. Both angiotensin II, via AT1 receptor, and the availability of pro-inflammatory cytokines increase cytosolic calcium concentration through the activation of phospholipase A2, which leads to inositol 3-phosphate production and, subsequently, calcium release from the endoplasmatic reticulum. Elevated cytosolic calcium concentration induces protein kinase C activation that phosphorylates serine 303, 304 and 328 autoinhibitory domains of p47phox, a regulatory subunit of NADPH oxidase. These post-translational modifications allow p47phox migration from cytosol to cell membrane, where it is assembled to others NADPH oxidase's subunits, as the catalytic subunit gp91phox, releasing superoxide anions [44, 50, 58, 59] and causing neuronal activation.

In addition to increase the neuronal activity, reactive oxygen species also stimulate redox-sensitive signaling pathways, the mitogen-activated protein kinases (MAPK). In the PVN and RVLM angiotensin II induces p42/44 MAPK phosphorylation therefore increasing the activity of several transcriptional factors, such as CREB, AP-1 and NF-kB. Upregulation of these transcriptional factors trigger a positive feedback mechanism between oxidative stress, inflammation and angiotensin II, since it increases gene expression of NADPH oxidase's subunits, pro-inflammatory cytokines (Tumor necrosis factor-alpha, Interleukine-6 and Interleukine-1beta) as well as the gene expression of RAS's components (angiotensinogen, angiotensin converting enzyme and AT1 receptor), amplifying the deleterious effect on autonomic areas.

In the NTS, reactive oxygen species are shown to attenuate NA' stimulation, since these neural connections are positively modulated by nitric oxide and the reactive oxygen species reduce nitric oxide availability by nitric oxide synthase uncoupling (see sections below). Within the PVN preautonomic neurons, reactive oxygen species reduce nitric oxide availability and, consequently, NR1 subunit of NMDA receptor nitrosylation, an important mechanism for the inactivation of local glutamatergic signaling. Thus, reactive oxygen species increase neuronal activity, leading to baroreflex dysfunction and sympathetic hyperactivity [45, 48, 60–62].

Directly related to the angiotensin II-induced neuronal inflammation, microglia activation was shown to be an important source for pro-inflammatory cytokines and reactive oxygen species in the brain of hypertensive individuals [63, 64]. In the neurogenic hypertension induced by chronic angiotensin II infusion or systemic inflammation, Shi et al. [64] and Wu et al. [65] have shown microglial activation within the PVN and RVLM, marked generation of pro-inflammatory cytokines and augmentation of both plasma norepinephrine content and blood pressure levels. In the PVN of the adult SHR we recently showed robust microglia activation and marked increase in the local synthesis of pro-inflammatory cytokines, which are

accompanied by reduced baroreflex sensitivity, increased sympathetic and reduced parasympathetic activity and increased blood pressure variability, important markers of autonomic dysfunction [66].

2.2 *Correction of Autonomic Dysfunction by Exercise Training*

Aerobic exercise training is recognized as one of the most efficient non-pharmacological therapeutic strategies, producing in hypertensive patients an arterial pressure reduction in the range of 8–5 mmHg [67, 68]. In the 3-months old SHR, 6–8 weeks of moderate aerobic exercise training (5 sessions per week, 1 h per session and 50–60% of maximum exercise capacity) are able to significantly decrease mean arterial pressure by approximately 5–15% [3, 46, 69, 70]. However, pressure fall is partial and pressure levels are still higher in the trained SHR when compared to sedentary age-matched normotensive controls. Similar data were observed in the renovascular model of hypertension [71, 72].

Besides the beneficial remodeling of the microcirculation in exercised tissues (capillary angiogenesis, wall/lumen ratio normalization of the hypertrophied arterioles, increased conductance of small venules, [73–77]), training-induced regression of autonomic dysfunction is considered one of the most important mechanism for the correction of hypertension-induced deleterious adjustments, in addition to the partial blood pressure fall. In the adult SHR only 1–2 weeks of aerobic training are able to downregulate brain RAS [2] and to reduce oxidative stress and inflammatory profile in brain autonomic areas [3, 66], therefore normalizing baroreflex control of the heart simultaneously with the reduction of pressure variability and augmentation of heart rate variability [2, 3, 66]. These autonomic benefits of exercise training occur before the appearance of resting bradycardia and pressure fall (~5–8%, usually around the 4th week of training) and are significantly correlated with the reduction of angiotensinogen expression in the PVN [2, 3, 66]. Normalization of baroreflex function associated to increased vagal cardiac activity contributes to resting bradycardia in hypertensive-trained rats. As a consequence, 2-weeks trained SHRs present a near normal autonomic control, reduced sympathetic and elevated cardiac vagal activity, even exhibiting high-pressure levels [2, 3]. Improvement of reflex bradycardia and decreased level of both oxidative stress and pressure were also observed in the left ventricle and kidney of renovascular hypertensive rats submitted to 4 weeks of swimming training [71].

Previous experiments from our and other laboratories have shown that aerobic training in SHR increases the sensitivity of aortic baroreceptors [78], augments the density of noradrenergic ascending projections from NTS to preautonomic neurons in the PVN [79], induces plastic changes and increases the density of oxytocinergic neurons within autonomic PVN subnuclei [80, 81], augments the intrinsic excitability of these preautonomic neurons projecting to brainstem areas involved in the

primary integration of baroreceptors reflex [82], increases density of oxytocinergic projections to the NTS/DMV area and the local synaptic release of oxytocin [7, 15], thus facilitating the vagal outflow and the appearance of resting bradycardia [7, 80] as well as the slowdown of the heart of trained rats during submaximal exercise [83]. These effects were abolished by sinoaortic denervation or oxytocin receptor blockage in the NTS [84–86]. Taken together, these data demonstrate that increased pressure during repeated daily exercise sessions activates baroreceptors and the supramedullary modulatory oxytocinergic pathway that increases the parasympathetic control of the heart.

Together with this effect, data from our laboratory also showed marked reduction of sympathetic vasomotor activity in the trained SHR, a response completely blocked by sinoaortic denervation [85]. Knowing that the generation of sympathetic activity involves the activation of excitatory glutamatergic neurons in autonomic areas and that oxidative stress and inflammation are potent activators of neuronal discharge [41–46, 50, 87], next we evaluate the effects of exercise training on the availability of reactive oxygen species and pro-inflammatory cytokines within the PVN. We observed in the trained SHR a prompt (2 weeks) and marked reduction in the expression of different NADPH oxidase subunits, normalization of the oxidative stress, decrease of p42/44 MAPK phosphorylation and NF-kB transcriptional activity, with a great reduction of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFα) expression in the PVN [3]. These responses were accompanied by decreased neuronal activity within this area as documented by Stern et al. [88]. Again, trained-induced changes in the PVN occurred simultaneously with normalization of baroreceptor reflex control of heart rate and preceded the appearance of resting bradycardia and pressure fall [3]. Training-induced blockade of oxidative stress, attenuation of the inflammatory profile accompanied by reduction of sympathetic nerve activity and a partial blood pressure fall are also observed in other autonomic areas, such as the RVLM [46, 89].

Besides the direct effect on pro-inflammatory cytokines expression, aerobic exercise training is also able to normalize high mobility group box protein 1 (HMGB1) availability in the PVN of the trained SHR. HMGB1, a damage-associated molecular pattern, acts through toll-like receptor 4 (TLR4) or CXCR4, a chemokine receptor type 4 in microglial cells, promoting pro-inflammatory cytokines expression and autonomic dysfunction [66]. Interestingly, 2-weeks of exercise training reduces the expression of both HMGB1 and CXCR4, normalizes the elevated translocation of NF-kB to the nucleus, restores the activated microglia to the inactive state, normalizes protein expression of TNFα and IL-6 in the PVN of the SHR, reduces pressure variability and corrects the autonomic dysfunction without any change in pressure levels [66].

Since the most important factor to avoid end-organ damage and mortality in hypertensive individuals is the reduction of pressure variability, the above-mentioned findings revealing a close relationship between training-induced cellular/molecular mechanisms and autonomic benefits show that training is able to interrupt the deleterious positive feedback between hyperactive brain RAS, oxidative stress and

inflammation observed in autonomic areas. Indeed, several experimental studies revealed reduced end-organ injuries in trained hypertensive animals. For instance, 8 weeks of aerobic training attenuate myocardial collagen accumulation and fibrosis in adult [69, 90] and aged SHR [22], contributing to restore the depressed diastolic function. Also, training decreases cytosolic calcium concentration, attenuates calcineurin-NFAT pathway and decreases left ventricle wall thickness, therefore correcting the deleterious hypertension-induced cardiac remodeling [69, 90]. The functional benefits of exercise training are also associated to the decrease of either NADPH oxidase-generated superoxide, NF-kB activity and pro-inflammatory cytokines expression in the heart, kidney and brain, thus contributing to reduce the local end-organ damage in hypertensive animals [46, 69, 91].

3 Mechanisms Contributing to Deleterious Remodeling of Peripheral Circulation

Drawing up the pathophysiological picture of hypertension, vascular deleterious remodeling contributes to establishment and maintenance of essential or primary hypertension [92, 93]. Vessels' adaptations in hypertensive subjects occur in different segments of the vascular tree: stiffness in conducting and muscular arteries, marked hypertrophy in small arteries and arterioles and capillary/small veins rarefaction in the microcirculation. Experimental studies demonstrated that increased arterial stiffness (defined as the decreased capacity of the vascular wall to convert kinetic energy in elastic potential energy) precedes the onset of blood pressure elevation in salt-sensitive [94], essential [95] and high fat diet hypertension [96]. Two well-characterized mechanisms promote arterial stiffness in hypertension: reduction of fenestrae's density in the internal elastic lamina and disorganization of the vascular tissue. Juvenile pre-hypertensive SHRs (30 days-old) already exhibit decreased fenestrae area and elastin deposition when compared to age-matched Wistar-Kyoto rats. These two structural factors promote a left shift of stress x strain curve, a mechanical abnormality that increases wall stiffness in conductance vessels of the juvenile SHRs [97, 98].

Vascular tissue disorganization results from cellular/molecular dysfunctions in endothelial and smooth muscle cells. In hypertensive subjects, endothelium presents marked imbalances between vasodilator and vasoconstrictor factors, antioxidant enzymes and pro-inflammatory agents. The major endothelial molecular mediator, the nitric oxide (NO), is produced in a reaction catalyzed by endothelial NO synthase (eNOS). This reaction converts L-arginine into L-citrulline, releasing nitric oxide, NADP+ and a water molecule. Tetrahydrobiopterin (BH4) is a important co-factor for nitric oxide production. In a hypertensive endothelial environment, BH4 is oxidized to dihydrobiopterin (BH2, a biologically inactive form) by the abundant reactive oxygen species, such as superoxide and hydrogen peroxide. In the absence of BH4, eNOS still releases NO, but also superoxide, which reacts with NO producing the peroxynitrite (eNOS uncoupling). In addition to BH4 oxidation,

endothelial cells from hypertensive subjects exhibit increased expression of inducible NO synthase (iNOS), which is calcium independent and, consequently, presents a higher activity when compared to eNOS. Therefore, iNOS reacts with BH4 and reduces its bioavailability for eNOS, increasing its uncoupled state [99].

Similar to the effects observed in the brain, the presence of reactive oxygen species, pro-inflammatory cytokines and activated tissue renin-angiotensin system triggers a positive feedback mechanism in the vessel wall. Elevated reactive oxygen species inactivate protein tyrosine phosphatases through irreversible catalytic cysteine oxidation [100, 101]. This post-translational modification increases MAPK signaling pathway and the transcriptional activity of several factors [NF-kB, cAMP response element-binding protein (CREB) and activator protein-1 (AP-1)] that intensify gene expression of many pro-inflammatory cytokines, NADPH oxidase subunits and several RAS components amplifying both endothelial dysfunction and vascular remodeling.

In the smooth muscle cells of the arteriolar wall, Angiotensin II exerts direct vasoconstrictor and trophic effects. In smooth muscle cells isolated from rat arteries, angiotensin II modulates several types of ionic channels, activates protein kinase C and inhibits protein kinase A [102–104]. These effects inhibit voltage-gated K+, delayed rectifier K+ and ATP-sensitive K+ channels causing a subsequent vasoconstriction, which increase vasomotor tonus and the total peripheral vascular resistance [102–104].

In the conductance arteries, angiotensin II, via AT1 receptor signaling, also promotes monocyte chemotactic protein-1 (MCP-1) and transforming growing factor-β1 (TGF-β1) gene expression in smooth muscle cells, which act autocrinally through C-C chemokine receptor type 2 (CCR2) and type II TGFβ receptor (TβRII), respectively. These molecular signaling pathways increase MCP-1, matrix metalloproteinase-2 (MMP2), fibronectin and collagen [105–107]. Although collagen accumulation is identified in adult hypertensive animals, it is not observed in pre-hypertensive SHRs that already demonstrate increased arterial stiffness, excluding the causative role of collagen in arterial stiffness [97, 98]. MMP2 cleaves the latent TGF-β1 form (TGF-β1 associated with Latency Associated Protein) releasing TGF-β1 active form. MMP2 also cleaves pro-endothelin-1 to endothelin-1 active form, which mimics angiotensin II's molecular effects. Additionally to activating peptides, MMP2 is able to digest elastin and, consequently, induces internal elastic lamina fragmentation, all these effects contributing to increase arterial stiffness.

In hypertensive individuals, augmented arterial stiffness increases pulse wave velocity, facilitating the return of reflective waves to left ventricle during the systole, which increase the systolic pressure. Then, the myocardium has to increase ventricular pressure to overcome the higher resistance to left ventricle ejection. As a result, left ventricle hypertrophies [108], compromising its perfusion and leading to myocardium ischemia especially during elevated metabolic demand, as the submaximal exercise. Arterial stiffness-induced increased pulsatility also promotes smooth muscle cells hypertrophy in arterioles and a further increase in total peripheral resistance and the consequent elevation of diastolic arterial pressure [108].

3.1 Exercise Training in the Amelioration of Peripheral Circulatory Deficits

The peripheral vascular adaptations are the best well-documented exercise training benefits. Old experimental studies have already identified the improvement of both endothelial function in conductance vessels and capillary density in a variety of tissues [109, 110]. The main mechanism suggested to explain training-induced vascular benefits is the shear stress [111, 112]. During an acute bout of exercise, cardiac output raises in order to achieve the increased metabolic demand. As a consequence, the augmented frictional force generated by the increased blood flow triggers calcium influx from extracellular into the cytosol of endothelial cells through mechanosensing ion channels. Mechanical deformation of endothelial cells also increases the calcium flux from endoplasmatic reticulum to cytosol. Calcium not only is a required cofactor for eNOS catalytic effect, but also triggers calcium-calmodulin kinase activity, which phosphorylates eNOS and causes NO release. A further increase in cytosolic calcium is mediated through integrins' activation by the shear stress, which activates phosphatidylinositide 3-kinases/protein kinase B (PI3K/Akt) signaling pathway [113]. This signaling pathway directly phosphorylates eNOS, increasing NO production.

NO exhibits a wide variety of cellular/molecular actions, many of them modulating the benefic vascular remodeling induced by exercise training. NO diffuses from the endothelial membrane to smooth muscle cells, where it activates soluble guanylate cyclase increasing local cGMP levels. cGMP activates cGMP-dependent kinases that decrease cytosolic calcium, inducing the vasodilation. In addition to the direct vasodilator effect, NO attenuates NADPH oxidase activity through S-nitrosylation of p47phox. NO, via S-nitrosylation, also elicits the inhibition of the highly reversible protein tyrosine phosphatases protecting them from the irreversible cysteine oxidation and inactivation [114]. Taken together, these data indicate that NO inhibits the production of reactive oxygen species by the NADPH oxidase and induces potent vasodilation.

During the acute bout of exercise, endothelial cells produce reactive oxygen species, which oxidize a critical cysteine of Keap-1 (a Nrf2 repressor protein) causing the translocation of Nrf2 to the cellular nucleus where it binds to ARE (antioxidant responsive element) inducing gene expression of several antioxidant enzymes, such as heme oxygenase-1 and thioredoxin reductase-1 [115–117]. In the thoracic aorta and small mesenteric arteries training attenuates thromboxane A2-induced vasoconstriction and increased the vasodilation to acetylcholine in SHRs [118, 119] and obesity-induced hypertension [120]. Together, these mechanisms improve endothelial-induced reduction of the vasomotor tonus in arteries. Exercise intensity is shown to affect vascular response to training. Battault and colleagues [121] compared moderate vs. high intensity (55% vs. 80% of maximal exercise capacity) showing that high intensity induces oxidative stress (and the consequent eNOS

uncoupling), the endothelial function in SHR aortas being improved only by moderate exercise intensity. Similar results were observed in health individuals [122].

Both high NO bioavailability and increased antioxidant enzymatic system are proposed as the main mechanisms to reduce the expression of the vasoconstrictor RAS axis and attenuate angiotensin II effects in vessel wall. In fact, gene and protein expression of angiotensinogen, angiotensin converting enzyme and AT1 receptor are decreased in the aorta of trained SHR [118], training also attenuated angiotensin II's vasoconstrictor effects in aorta of ovariectomized spontaneously hypertensive rats independently of estrogen therapy [123]. Sequential measurements of angiotensinogen expression (western blotting) and of the content of angiotensin II and angiotensin (1–7) in the renal, femoral, carotid and thoracic aorta (high performance liquid chomatrography) in adult SHR submitted to aerobic training revealed that only 1–2 weeks are able to normalize the elevated angiotensinogen content in the renal artery, which is accompanied by a parallel robust reduction of angiotensin II concentration and a mild decrease in angiotensin-(1–7) content in renal artery [70]. The differential responses of the vasoconstrictor and vasodilator RAS axes result in a complete normalization of the angiotensin II/angiotensin-(1–7) ratio in the renal arteries of the SHR at the 4th week of training, coinciding with the partial but significant decrease in arterial pressure (5–6%). These vascular changes are accompanied by similar responses of the intra-renal RAS axes [70]. In the other SHR arteries, RAS expression is also depressed but training-induced decreases are smaller and similar for both angiotensin II and angiotensin-(1-7) with unchanged vasoconstrictor/vasodilator ratio within the femoral, carotid and thoracic aortas [70]. Together these results showing similar training-induced time course RAS changes for peripheral tissues and brain (previous section) indicate a broad and prompt response to exercise in order to overcome the deleterious circulatory and autonomic responses triggered by hypertension; they also suggest a wide effect of aerobic training to downregulate both RAS axes, in order to maintain its equilibrium in a lower level. The higher expression of angiotensin II and angiotensin-(1-7) in renal arteries of sedentary SHR (over 30-fold when compared to other territories, [70]) and the its marked reduction in trained rats confirm the important role of kidney RAS changes in both the development as well as the regression of deleterious hypertension-induced changes. Exercise training is also able to correct in the vasculature the cellular responses associated with RAS hyperactivity as the oxidative stress and pro-inflammatory profile [70, 118, 119].

Long-term exercise training (12 weeks) is effective in normalizing collagen accumulation, MMP9 expression and fenestrae density therefore correcting the stress x strain relationship in coronary and mesenteric arteries from SHR [119]. Also, trained SHRs present intact internal elastic lamina and attenuated collagen gene expression in the aorta [124]. Although based on associative data, normalization of mechanical properties (which reduces pulse wave velocity and pulsatility in hypertensive arteries, [119]) seems to be related with cardiovascular benefits observed in trained rats.

Aerobic training is also able to reduce total peripheral resistance by normalizing the vascular resistance of exercised tissues. A complete regression of increased arteriolar wall/lumen ratio in skeletal muscles, myocardium and diaphragm is observed in trained SHRs, the decrease in arterioles wall/lumen ratio being positively correlated with the reduction in both skeletal muscle vascular resistance and blood pressure [73–75, 77, 125]. Data from these studies showing unchanged arterioles wall/lumen ratio in tissues that respond with vasoconstriction to acute bouts of exercise thus maintained elevated local vascular resistance [73, 77] highlighted why training reduce but does not normalize blood pressure levels. These vascular adaptations combined with improved autonomic control and decreased sympathetic outflow, contribute to decrease the vascular response during lumbar nerve stimulation, muscle contraction and dynamic submaximal exercise in trained hypertensive rats [75, 126, 127]. Indeed, exercised-muscles in old trained rats exhibit attenuated angiotensin II-induced vasoconstriction when compared to age-matched sedentary controls [128].

Besides arterial and arteriolar adaptations, exercise training also increases the density of small venules (cross-sectional area < 300 μm) in skeletal muscles [74, 76] and causes robust capillary angiogenesis in trained hypertensive animals [73–77, 109, 129–132]. VEGF is recognized as the main molecular player in training-induced angiogenesis and is rapidly (~3 days) activated by exercise training [129, 130]. Post-transcriptional regulation by miRNAs is also involved in the vascular response to training: compared to sedentary hypertensive controls swimming-training reduced the increased expression of miRNAs-16 and -21, and increased that of miRNA-126 [130]. miRNA-16 and -126 interact directly and regulate the activity of VEGF and PI3KR2 (a negative regulator of PI3K/Akt/eNOS pathway), respectively [133, 134]. In agreement with miRNAs' changes, swimming-trained hypertensive rats exhibit increased VEGF and eNOS protein levels, inhibiting capillary apoptosis and restoring its density [130]. In fact, additional rise of capillary/fiber ratio contributes to hypotensive additive effect in angiotensin-converting enzyme inhibitor treated and trained hypertensive rats [131, 132].

4 Conclusions

Development of hypertension in different experimental models is accompanied by unbalance of the renin-angiotensin system, oxidative stress and inflammation that trigger several autonomic and peripheral deficits. These deleterious hypertension-induced cardiovascular deficits condition end-organ damage being important risk factors for increased morbimortality in hypertensive subjects. As summarized in Fig. 16.1, experimental studies provided extensive data demonstrating that moderate-intensity exercise training is a crucial therapeutic tool to overcome most of the deleterious hypertension-induced effects. It has potent and wide effects counteracting/normalizing the cellular/molecular pathological mechanisms induced by

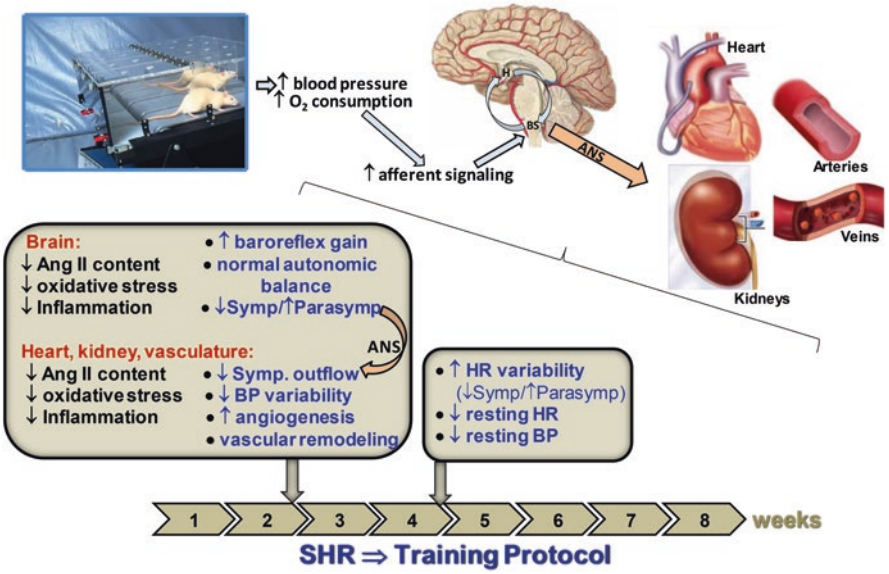

Fig. 16.1 Scheme depicting beneficial training-induced effects on brain and peripheral tissues. Repetitive acute bouts of exercise (aerobic training) increases blood pressure (*BP*) and oxygen (O_2) consumption, activating arterial baroreceptors and chemoreceptors. Increased afferent signaling activates NTS barosensitive and chemosensitive neurons in the brainstem (*BS*), which project to preganglionic parasympathetic and premotor sympathetic neurons within the BS, besides projecting to integrative areas within the hypothalamus (*H*) that continuously modulate primary BS integration of the afferent signaling. As a result, autonomic nerve system (*ANS*) firing pattern changes increasing the parasympathetic and decreasing the sympathetic outflow to the periphery, therefore correcting the autonomic deficits exhibited by spontaneously hypertensive rats (*SHR*). Functional and neurohormonal time course changes within the brain, heart, kidneys and vasculature during the training protocol as well as the main cellular mechanisms conditioning the benefic responses induced by aerobic exercise training are depicted in the *bottom part* of the figure

hypertension not only in the brain, but also in peripheral tissues. Trained hypertensive individuals show a normal autonomic balance, improved control of the circulation and reduced both end-organ injuries and cardiovascular mortality.

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Chapter 17
Exercise Training in Pulmonary Hypertension and Right Heart Failure: Insights from Pre-clinical Studies

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Abstract Exercise training (ExT) is widely used for the prevention and treatment of several chronic cardiovascular diseases. However, only recently it started to be recognized as safe and beneficial in pulmonary arterial hypertension. Despite the consistency of its favorable effects on exercise tolerance and quality of life, the mechanisms underlying these meaningful clinical improvements remain unclear. Current studies emphasize the exercise-induced changes on skeletal muscle but the impact of ExT at the level of the pulmonary circulation and right ventricle should not be overlooked. In this chapter, we summarize the main findings from pre-clinical studies analyzing the impact of exercise in pulmonary hypertension and right heart failure.

Keywords Exercise • Cardiovascular diseases • Pulmonary arterial hypertension • Right ventricle

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1 Introduction

Pulmonary arterial hypertension (PAH) is a disorder of the pulmonary circulation and it is defined by hemodynamic criteria: a mean pulmonary arterial pressure (PAPm) ≥ 25 mmHg at rest, a pulmonary artery wedge pressure < 15 mmHg and a pulmonary vascular resistance (PVR) > =3 Wood units [1]. It is characterized by a gradual increase in PVR reflecting the progressive obliteration of small pulmonary arteries. Consequently, PAH increases the right ventricle (RV) afterload, resulting in maladaptive remodeling and failure, leading to premature death [2]. PAH may arise in association with a broad range of diseases and its prevalence is estimated to be 10–15 cases per million with a mortality rate of 15% per year but information may differ through registries [1, 3]. Regardless of the etiology, symptoms are non-specific and mainly reflect the deterioration of the coupling between the RV and pulmonary circulation. They can include shortness of breath, fatigue, weakness, angina and syncope [1]. Over the past two decades, advances in PAH-specific therapies have improved survival and slowed disease progression [4–6]. However, most patients remain symptomatic with significant exercise intolerance, reduced quality of life and still have an ominous prognosis.

Exercise training (ExT) has preventive and therapeutic effects in several chronic diseases [7, 8] but only recently it started to be recognized as safe and beneficial in PAH. In fact, PAH treatment guidelines used to advise that any physical activity should be limited as it could aggravate the disease progression and increase the risk of sudden cardiac death [9]. However, accumulating evidence suggests a positive effect of supervised ExT in functional capacity and quality of life, when added to the best standard of care with approved medications. Importantly, ExT seems to have a reassuring safety profile [10, 11]. The physiological mechanisms that explain the increased exercise tolerance attained by PAH patients enrolled in structured ExT programs are still unclear. Beneficial changes in cardiac output, PVR, chronotropic response to exercise and peripheral skeletal muscle have all been described [10]. Accordingly, current guidelines now recommend that PAH patients should be encouraged to be active within symptom limits and, when physically deconditioned,

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they should undertake supervised ExT under medical therapy [1]. Despite this major change regarding the role of exercise in the management of PAH patients, the mechanisms underlying these clinical improvements remain unclear. In this chapter, we will summarize the main findings from pre-clinical studies analyzing the impact of exercise in PAH and right heart failure.

2 Pre-clinical Models to Study the Impact of Exercise Training in Pulmonary Hypertension and Right Heart Failure

There are various pre-clinical models of PAH based on physical, chemical, genetic or a combination of insults that had been useful in the last decades to study both the impact of drugs and non-pharmacological interventions such as exercise. None of them fully recapitulates all features of human PAH and they exhibit specific advantages and limitations described elsewhere [12–14]. The most commonly used PAH pre-clinical models to study the effects of ExT are monocrotaline (MCT; 12 studies) and chronic hypoxia (4 studies). Of note, these models also grounded the development of therapies currently available for this condition [13]. The MCT model mimics human PAH in terms of hemodynamic and histopathological severity, and high mortality; it differs on the early presentation of lung edema, loss of the endothelial barrier and prominent inflammatory adventitial proliferation [15]. The phenotypical changes induced by MCT are dose-dependent (60 mg/kg for severe PAH or 30 mg/kg for stable PAH) and only require one single administration (subcutaneous or intraperitoneal). Signs of illness start to occur within 3–7 days, with animals presenting anorexia, failure to gain weight and tachypnea [15]. As lung injury and vascular remodeling progresses, animals develop variable degrees of dyspnea, weakness, diarrhea, and peripheral cyanosis. PAPm is increased 2 weeks after MCT injection, leading to RV hypertrophy by the third week. By 5–6th week, half of the injected rats usually die [15]. PAH due to chronic hypoxia model consists on exposing animals to normal air at hypobaric pressure or to oxygen-poor air at normal pressure [16]. The decrease in oxygen pressure causes a strong pulmonary vasoconstrictor response followed by progressive hypertrophy (but little proliferation) and muscularization of medial pulmonary arterioles, endothelial dysfunction and a doubling of PAPm [13]. A proinflammatory microenvironment capable of promoting recruitment, retention and differentiation of circulating monocytic cell populations, possibly contributing to vascular remodeling, has also been described [17]. Hypertrophy of the RV occurs just after 2 weeks of exposure to chronic hypoxia but RV failure, which is the main cause of death in PAH patients, does not occur in this model [17].

3 Exercise Training and the Right Heart in Pulmonary Arterial Hypertension

The primary cause of death in patients with PAH is right heart failure, which is preceded by RV dysfunction [18]. Right heart failure can be reversed by interventions that normalize RV afterload such as lung transplantation and pulmonary endarterectomy but there is no specific therapy directly targeting RV. If available, such strategy could have tremendous clinical implications because RV function is a major independent prognostic determinant of PAH patients [18]. Aerobic exercise training was shown to improve cardiac function and reverse ventricular remodeling in clinically stable individuals with left heart failure and left ventricular (LV) systolic dysfunction [19]. Despite the intimately related function of both ventricles, there are striking differences about how they respond and adapt to physiological or pathological stimuli [20–22]. In addition, the response of pulmonary and systemic circulation to exercise is also very different. Together, these distinctive features preclude any recommendation of ExT to PAH patients based on the evidence from LV failure. While the data derived from clinical studies is scarce [23], the first insights about the impact of ExT in RV function and remodeling came from pre-clinical studies.

3.1 Exercise Training and Right Ventricular Function

A detailed characterization of training programs, animal models and changes induced by ExT in PAH is provided in Tables 17.1, 17.2 and Fig. 17.1. Overall, studies differ in terms of MCT's concentration, animals' weigh, age and species, exercise intensity and duration, and time point of the disease when ExT was initiated. The majority of the studies argue that ExT can prevent RV systolic [24–32] and diastolic dysfunction [25, 27, 29] while a minority shows no change (nor beneficial nor deleterious) [30, 33, 34], and two studies report aggravation [33, 35]. RV function was assessed by a variety of invasive and non-invasive parameters such as cardiac output (CO), stroke volume (SV), fractional shortening (FS), myocardial acceleration during isovolumic contraction (AIV), isovolumic relaxation time (IVRT), tricuspid annular plane maximal systolic velocity (E'), tricuspid annular plane systolic excursion (TAPSE), end-diastolic pressure (EDP), time constant of ventricular pressure decay (Tau), end-diastolic (EDPVR) and end-systolic pressure-volume relationship (ESPVR). Those studies reporting enhancement of RV function have in common the use of higher exercise intensities [25, 27–30, 32], suggesting that the benefit may be intensity-dependent. Similarly, it is accepted that training-related cardiac adaptations to the LV are dependent on training intensity [36–38]. Regarding the RV, this hypothesis was specifically evaluated in one study, where high intensity interval training (HIIT), but not continuous aerobic training, was able to improve RV cardiac index [30]. Likewise, it is interesting to note that free wheel running that is characterized by intermittent, high intensity but short bouts of running throughout the day [39],

Table 17.1 Characterization of training programs used to evaluate the impact of exercise training in pre-clinical models of Pulmonary Arterial Hypertension

References	Mode of exercise		Intensity	Session duration (min)	Days per week	Total duration (weeks)
[35]	AET	FT	0.9 km/h	60	5	2
		TP	40% Vmax	60	5	3
[34]	AET[1]	FT	15 cm/s	5	3	2
		TP	50% Vmax, −15° slope	30	5	4
[33]	AET	FT	13.3 m/min	1	5	3
		TP	13.3 m/min	30	5	4
[30]		FT	6–15 m/min, 0–15° slope	5	5	5
	HIIT	TP	5x(2 min at 85–90% VO_2R and 3 min at 30% VO_2R)	30	5	6
	AET	TP	50% VO_2R	60	5	6
[26]	AET	FT	0.6 km/h–0.9 km/h	15–60	5	2
		TP	60% VO_{2max}	60	5	3
[32]	AET	FT	0.6 km/h–0.9 km/h	15–60	5	2
		TP	60% VO_{2max}	60	5	3
[25]	AET	FT	0.6 km/h–0.9 km/h	15–60	5	2
		TP	60% VO_{2max}	60	5	3
[28]	FWR	TP	Free access to running wheel	–	7	4
[31]	AET	FP	Progressive increase from 0.6 km/h to 0.9 km/h	15–45	5	2
		TP	Progressive increase until 1.1 km/h by the 10th week; 0.8 km/h from 11th to 12th week; 0.9 km/h in 13th week	60	5	11
[86]	AET	FT	NA	NA	5	2
		TP	0.6 km/h–0.9 km/h	50	5	5
[27]		FT	20 m/min	20–60	5	1
	AET	TP-early	30 m/min	60	5	4
	AET	TP-late	30 m/min	60	5	2
[29]	AET	FT	20 m/min	20–60	5	1
		TP	25 m/min	60	5	4
[81]	AET	TP	30 m/min, 10° slope	60	5	10
[24]	AET	TP	30 m/min, 10° slope	60	5	5
[85]	AET	TP	80% of Vmax	60	5	5
[84]	AET	TP	60% Vmax	30	5	3

FT familiarization to treadmill; *TP* training protocol; *HIIT* high intensity interval training; *AET* continuous aerobic *ExT*; *AET*[1] downhill (negative slope) aerobic *ExT*; Vmax maximal running speed; *VO_2R* oxygen consumption reserve; *NA* information not available

Table 17.2 Characterization of pre-clinical models of Pulmonary Arterial Hypertension and summary of the main changes induced by exercise training

PAH	Species and weight or age[a]	Mode of exercise	Cardiac afterload[b]	Cardiac function[c]	Cardiac hypertrophy	PA hypertrophy	References
MCT (60 mg/kg)	MWR, ~100 g	AET	⇑	⇓	⇔	NA	[35]
MCT (40 mg/kg)	MWR, ~224 g	AET[1]	⇔	⇔	⇔	NA	[34]
MCT (40 vs. 60 mg/kg)	MWR, 150–175 g	AET	⇔⇑[2]	⇔⇓[4]	⇔	⇔⇑[2]	[33]
MCT (40 mg/kg)	MSDR, ~300 g	HIIT vs. AET	⇓⇔[3]	⇑⇔[5]	⇓⇔[6]	⇔	[30]
MCT (60 mg/kg)	MWR, ~146 g	AET	⇔	NA	⇔	NA	[26]
MCT (60 mg/kg)	MWR, ~315 g	AET	⇔	⇑	⇔	NA	[32]
MCT (60 mg/kg)	MWR, ~139 g	AET	⇔	⇑	⇔	⇓	[25]
MCT (60 mg/kg)	MWR, ~200 g	FWR	NA	⇑	⇓	NA	[28]
MCT (60 mg/kg)	MWR, ~206 g	AET	⇔	⇑	⇓	NA	[31]
MCT (60 mg/kg)	MWR, 160–180 g	AET	NA	NA	⇔	NA	[86]
MCT (60 mg/kg)	MWR, 180–200 g	AET	⇔	⇑	⇔	⇔	[27]
MCT (60 mg/kg)	MWR, 150 g	AET	⇔	⇑	⇓	⇓	[29]
Hypoxia (PIO_2 = 110 Torr)	MSDR, 10 weeks	AET	NA	NA	⇓	NA	[81]
Hypoxia (PIO_2 = 70 Torr)	MSDR, 200–225 g	AET	⇔	⇑	⇓	NA	[24]
Hypoxia ($PIO_2 \approx$ 90 mm hg)	MWR, 300–350 g	AET	NA	NA	⇔	NA	[85]
Hypoxia (10% O_2)	MC57BL/6 J	AET	⇓	NA	⇔	⇓	[84]

NA information not available; *MWR* male Wistar rats; *MSDR* male Sprague-Dawley rats; MC57BL/6 J, male C57BL/6 J mice; AET continuous aerobic exercise training; *HIIT* high intensity interval training; *FRW* free wheel running; ⇑, increased in comparison to sedentary with PAH; ⇔, no change in comparison to sedentary with PAH; ⇓, decreased in comparison to sedentary with PAH

[1]Downhill running; [2]No change in "stable PAH" but increased in "progressive PH"; [3]Decreased with HIIT and no change with AET; [4]No change in "stable PAH" but decreased in "progressive PAH"; [5]Increased with HIIT but no change in AET; [6]Decreased with HIIT but no change with AET

[a]Weight or age at the beginning of the study; [b]Cardiac afterload denotes changes in one or more of the following parameters: pulmonary vascular resistance (*PVR*), right ventricular systolic pressure (*RVSP*), pulmonary arterial pressure (*PAP*), arterial elastance (Ea), pulmonary artery acceleration time (PAAT) or acceleration time to ejection time ratio (AT/ET);[c]Cardiac function denotes changes in one or more of the following parameters: cardiac output (*CO*), stroke volume (*SV*), fractional shortening (*FS*), myocardial acceleration during isovolumic contraction (AIV), isovolumic relaxation time (*IVRT*), tricuspid annular plane maximal systolic velocity (E′), tricuspid annular plane systolic excursion (*TAPSE*), end-diastolic pressure (*EDP*), time constant of ventricular pressure decay (*Tau*), end-diastolic (*EDPVR*) and end-systolic pressure-volume relationship (*ESPVR*)

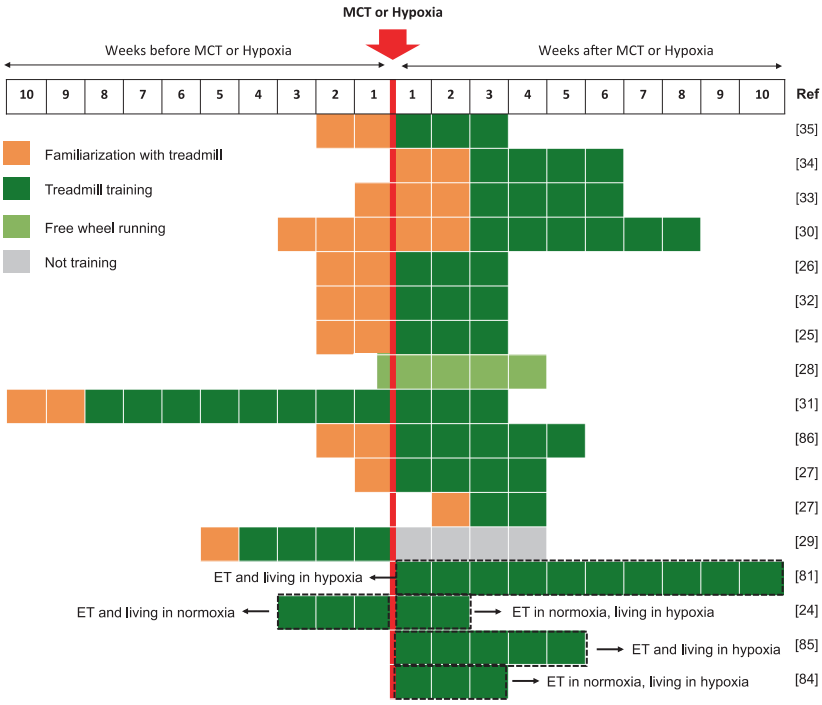


Fig. 17.1 Distribution of exercise sessions in relation to the time point where the stimulus for Pulmonary Arterial Hypertension was induced

was able to delay the onset of RV failure [28]. The impact of ExT on the cardiovascular system is also dependent on the exercise modality [40]. While concentric ExT (running in a treadmill with a slope ≥0°) conferred cardiac protection in different studies using either MCT or hypoxia, it seems that eccentric exercise (running in a treadmill with a negative slope), despite being safe in PAH, does not improve RV function [34]. Future studies should address the impact of different modalities and intensities in order to determine which exercise program provides better benefits.

Another important aspect to consider is the time point where ExT is initiated. Table 17.2 and Fig. 17.1 show that while starting ExT before [24, 29, 31] or at early disease stages of PAH [25, 27, 32] may be required for maximal benefits, beginning in latter stages may limit [27] or even worsen cardiac function [33]. Three possible implications emerge from these observations. First, ExT can be useful as a preventive strategy for the management of the disease since its early diagnosis and could be prescribed to those patients with less severe hemodynamic derangement and right ventricle dysfunction. Second, this could be particularly important for those at increased risk, such as in the familial form of PAH. The familial form is inherited as an autosomal dominant trait and is associated with a pattern of "genetic anticipation," a worsening of disease in subsequent generations, manifested by greater severity or earlier onset [9]. Finally, clinical trials looking at the impact of exercise

in PAH should enroll patients in different stages of the disease, so that we could have solid evidence over the entire spectrum of the disease.

The current strategy to preserve RV function in PAH is by attempting to reduce the RV afterload. This strategy is effective when loading conditions can be normalized, as it is the case with lung transplantation or with pulmonary endarterectomy in PAH and chronic thromboembolic pulmonary hypertension (CTEPH), respectively [41]. However, in a subset of patients, it was noted that RV dysfunction might progress despite a reduced PVR with targeted medical therapies. A deterioration of RV function was associated with poor outcome, irrespective of any changes in PVR [41]. Similarly, Eisenmenger syndrome [42] and congenital pulmonary stenosis [43] patients usually present a severe increase in RV chronic pressure-overloaded and a relatively good prognosis explained by adaptive remodeling of the RV to those burdensome hemodynamic conditions [44, 45]. The beneficial effects of ExT on RV function were not always mirrored by a reduction in RV afterload. As illustrated in Table 17.2, except for one [30], all studies showed an improvement of cardiac performance despite the presence of persistent RV pressure-overload [24, 25, 27, 29, 31, 32]. Measures of RV overload ranged from PVR, PAP, RV peak systolic pressure (RVSP), pulmonary artery acceleration time (PAAT), acceleration time to ejection time ratio (AT/ET) and, less frequently, arterial elastance (Ea). This unrelated change between RV afterload and RV function in response to ExT further strengthen the hypothesis that other factors, beyond afterload, are important modulators of RV function in PAH [46, 47]. More importantly, ExT seems to influence some of those factors and, consequently, might be used to increase tolerance to an increased afterload.

3.2 Exercise Training, Right Ventricular Hypertrophy and Remodeling

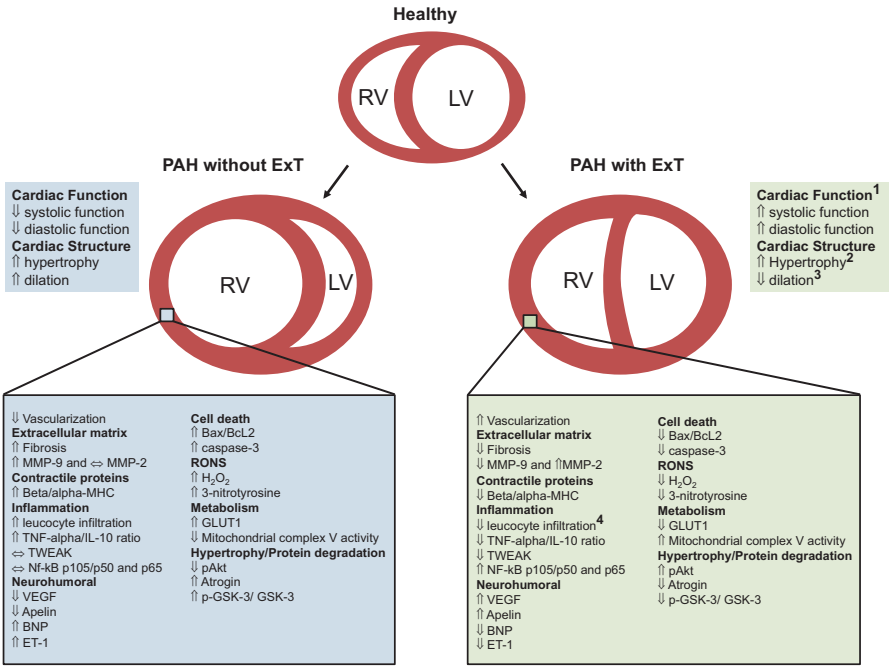
RV hypertrophy is a compensatory mechanism that modulates RV function within the homeostatic range. Following the law of Laplace, increased RV wall thickness (concentric hypertrophy) lowers RV wall stress and, together with changes in muscle properties, improves pumping effectiveness [18]. This compensatory stage is called “adaptive remodeling”. However, if the disease progresses, the hypertrophic process will be halted and CO falls [18]. In an attempt to restore CO, the RV dilates (eccentric hypertrophy) and heart rate (HR) increases, leading to RV uncoupling and reduced output in advanced stages of disease [48]. Thus, while RV dilatation might be beneficial in the acute phase (Frank-Starling mechanism), it will lead to increased RV wall stress, energy exhaustion, reduced RV function and failure in the long term [18]. This failing stage is called “maladaptive remodeling” [18]. As shown in Table 17.2, RV hypertrophy was not changed with ExT in 11 studies, which together with the improved RV function, suggests that ExT was capable to promote adaptive RV remodeling (or delay maladaptive remodeling). Further corroborating this notion, it was reported in one study that ExT prevented the RV to develop a spherical

shape, the leftward septal bowing and the enlargement of the right atrium [27], all of which are classical features of maladaptive remodeling [48]. Of note, this effect was observed when the ExT program was started in early stages of PAH [27], while in the most severe stages it seemed to exacerbate RV dilation [33]. On the other five studies that we analyzed, the RV mass decreased with ExT, a change that apparently was related to a protective effect of exercise on the lung vasculature as pulmonary resistance [30] and pulmonary artery hypertrophy [29] were shown to be decreased.

There are several changes in the myocardium, caused by chronic RV pressure overload and/or by the effects of circulating factors released from the sick lung circulation, possibly contributing for the transition from adaptive to maladaptive remodeling. Excellent reviews about this topic have been published elsewhere [21, 49]. Briefly, inflammation [50, 51], alpha to beta myosin heavy chain (alpha/beta-MHC) shift [52, 53], apoptosis [54], neurohumoral activation [55, 56], oxidative stress [57–59], mitochondrial dysfunction [60, 61], impaired metabolism [62–64], and disturbed angiogenesis and capillary rarefaction [65] were all identified to be present in the failing RV of animals and/or patients with PAH. According to the data collected from pre-clinical studies, ExT may prevent or delay maladaptive remodeling by modulating these changes. An integrative illustration of the molecular pathways affected by ExT is provided in Fig. 17.2.

Exercise training, when initiated before [29] or at an early disease stage [27], lead to a normalization of the levels of myocardial fibrosis, which likely contributed to restore diastolic stiffness and filling pattern [66]. Moreover, it prevented metalloproteinase (MMP)-9 activity and promoted an increase of MMP-2 activity, which might have decreased the accumulation of fibrosis [29]. The antifibrotic effect of exercise can also be related to its anti-inflammatory properties. Exercise training was reported to reduce the expression of TNF-alpha/IL-10 and TWEAK, and to modulate downstream regulators of the NF-κB pathway in MCT-treated rats [27, 29]. Also, no evidence of tissue inflammatory cell infiltration or cell death was noted following an acute bout of exercise [67], chronic continuous aerobic exercise [26] or high intensity interval training [30] in MCT-induced PAH rats. However, in the more severe form of MCT-induced PAH, ExT seems to result in widespread leucocyte infiltration of the RV [33]. It will be important to disclose if this contrasting results are because RV wall stress was detrimentally elevated during the exercise bouts (and thus ExT should not be recommended at advanced stages) or because animals were all exercising at the same absolute workload (rather than at a relative exercise intensity) [67].

Endothelin 1 (ET-1) antagonism is a mainstay of the actual therapeutic algorithm for PAH and seems to attenuate deterioration of cardiac function [68–71]. MCT-trained animals exhibited down-regulation of ET-1 mRNA in the RV [27]. B-type natriuretic peptide (BNP) is a dynamic measurement of the degree of RV dysfunction in PAH [72] and its expression was favorably modulated by ExT [27]. Apelin is a potent inotropic, anti-apoptotic, anti-inflammatory and pro-angiogenic neurohumoral mediator [73], and its expression was increased in the RV of rats with PAH after HIIT [30]. Finally, ExT-induced neurohumoral modulation was evident by the prevention of vascular endothelial growth factor (VEGF) mRNA down-regulation



Legend:

[1] ExT seems to worsen RV function in severe decompensated PAH.

[2] ExT seems to worsen RV dilation in severe decompensated PAH.

[3] ExT was shown to decrease RV mass in a few studies, which is probably related to a decrease in pulmonary resistance and pulmonary artery hypertrophy. In the majority of the studies, RV hypertrophy remained increased after ExT.

[4] ExT induced an increase in RV leucocyte infiltration only in severe decompensated PAH.

Fig. 17.2 Summary of the main changes induced by Exercise Training in the Right Ventricle of animals with Pulmonary Arterial Hypertension

[1]ExT seems to worsen RV function in severe decompensated PAH

[2]ExT seems to worsen RV dilation in severe decompensated PAH

[3]ExT was shown to decrease RV mass in a few studies, which is probably related to a decrease in pulmonary resistance and pulmonary artery hypertrophy. In the majority of the studies, RV hypertrophy remained increased after ExT

[4]ExT induced an increase in RV leucocyte infiltration only in severe decompensated PAH

[27], which may have contributed to improve cardiac capillary density in MCT-induced PAH [32, 33].

Oxidative stress has been implicated in RV maladaptive remodeling and dysfunction [57, 74]. By decreasing hydrogen peroxide production (H_2O_2), ExT training modulated the apoptosis regulator BAX/B-cell lymphoma 2 (Bax/Bcl-2) and caspase-3, thus decreasing apoptotic signaling in RV myocardium of MCT rats [26]. As major sources of reactive oxygen and nitrogen species (RONS), mitochondria them-

selves, and particularly oxidative phosphorylation complexes, are highly susceptible to oxidative and nitrative damage [75]. ExT performed in early or late PAH happened to prevent protein nitration of mitochondrial complex V and restore its activity [27]. ExT also improved RV myocardial metabolism by preventing the shift from mitochondria-based fatty acid oxidation to glycolysis found in PAH [30]. This is important as the switch from aerobic to anaerobic metabolism that occurs with mitochondrial dysfunction is involved in the transition to maladaptive remodeling [49].

Similar to the LV, down-regulation of fast alpha–myosin heavy chain together with overexpression of slow beta-isoform is present in the pressure-overloaded RV, but its long-term consequences remain unknown [21]. RV remodeling with ExT was associated with higher expression levels of alpha-MHC isoform [27, 29], which is in line with the beneficial effects of exercise training previously reported in LV failure [76, 77]. Exercise training, in the form of preconditioning, prevented the MCT-related overexpression of atrogin-1 [29]. When activated, this prominent ubiquitin ligase controls degradation of proteins contributing to cardiac muscle wasting and ventricular dysfunction [78]. Moreover, ExT stimulated the activation of protein kinase B (Akt) [26] that is associated with improved contractile function, cytoprotection, and increased synthesis of normal contractile proteins and metabolic enzymes [79].

RV failure is also associated with abnormalities in calcium handling proteins, including ryanodine receptor (RyR) and Ca^{2+} ATPase of sarcoplasmic reticulum (SERCA2a). Expression levels of SERCA2a [27], but not RyR [31] were restored in MCT-trained animals, possibly contributing to preserve relaxation rate. In humans and animals with PAH and RV failure, alpha and beta-adrenergic receptors density is decreased, which limits their response to inotropic agents and impairs exertional contractile reserve [80]. Exercise training was shown to suppress the downregulation of alpha-1 adrenergic receptors, to attenuate beta-adrenergic receptors decrease, and to lower muscarinic acetylcholine receptors in the rat model of hypoxia-induced PAH, eventually correcting chronotropic incompetence [81].

4 The Impact of Exercise Training on Pulmonary Artery Structure and Function

It is clear that the different forms of pulmonary hypertension can present with a predominance of pulmonary arterial remodeling, vein remodeling or a mixed contribution of both. While PAH is a classical example of the former, pure pulmonary venoocclusive disease and pulmonary hypertension due to left heart dysfunction are characterized predominantly by venous remodeling [82]. Virtually all forms of pulmonary hypertension, including those caused by interstitial lung disease, thromboembolic, hypoxia, and sarcoidosis may involve elements of both arterial and venous remodeling [82]. Remodeling of pulmonary blood vessels comprises thickening of the intimal and/or muscular vessels and the presence of cells expressing smooth muscle specific markers in pre-capillary arterioles (distal muscularization), caused by proliferation and migration of pulmonary arterial smooth muscle cells (PASMCs)

and possibly cellular trans-differentiation (i.e., endothelial-mesenchymal transformation) [83]. In addition, severe forms of PAH often present with vaso-occlusive lesions, involving PASMCs, endothelial cells and possibly cells of non-vascular origin. The greatest influence on PVR comes from changes in small arterioles; however, decreased compliance (i.e., increased stiffness) in the elastic proximal pulmonary arteries may also contribute for RV afterload [83].

Current knowledge about the impact of ExT on pulmonary architecture and/or vascular function is far more limited than for RV. From the 16 studies addressing ExT in PAH, only 6 reported measurements of pulmonary artery thickness (Table 17.2). The hypertrophy of the arteries was found to be reduced [25, 29, 84], to suffer no significant changes [27, 30, 33], or to be aggravated [33] after ExT. The worst outcome occurred when ExT was performed in the setting of advanced disease [33]. Regarding

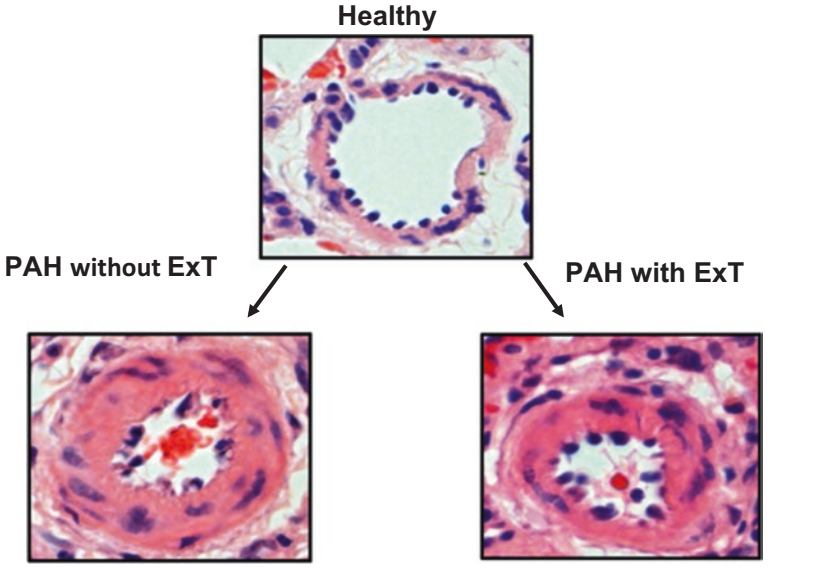


Fig. 17.3 Summary of the main changes induced by Exercise Training in the lungs of animals with Pulmonary Arterial Hypertension (**Note**: The impact of ExT on pulmonary artery wall thickness is inconclusive. *Arrows* in the *blue box* denote the direction of changes in comparison to sedentary healthy animals. *Arrows* in the *green box* denote the direction of changes in comparison to sedentary animals with PAH)

to respiratory functional parameters, higher PaO_2 and lung diffusing capacity at rest and during maximal exercise were described after ExT in hypoxia-induced PAH [24]. Concerning to vascular function, a single bout of exercise was able to transiently normalize PAP in MCT-induced PAH, revealing an exercise-induced "window" of pulmonary hypertension alleviation [67]. This effect was associated with increased lung nitric oxide synthase (eNOS) activation, supporting a mechanism of acute NO-mediated pulmonary vasodilatation [67]. A greater increase in total eNOS expression was also observed after chronic HIIT, paralleled by a decrease in total PVR [30]. In contrast, eNOS expression and activity was reduced in lung tissue homogenates after less intense but continuous ExT [30, 35]. The exposure of the pulmonary vasculature to a pulsatile flow-shear stimulus was proposed to explain the greater levels of eNOS obtained with HIIT [30]. In hypoxia-induced PAH, despite decreasing small pulmonary vessel muscularization, chronic exercise failed to properly modulate the nitric oxide synthase-soluble guanylyl cyclase-cyclic guanosine monophosphate-phosphodiesterase (NOS-sGC-cGMP-PDE) axis (at the mRNA level), in order to promote vasodilation [84]. Moreover, ExT failed to improve pulmonary artery vascular reactivity in hypoxia-induced PAH, as the responsiveness to vasoconstrictor (ET-1, epinephrine or potassium chloride) or vasodilator (acetylcholine or sodium nitro-prusside) substances remained increased and decreased, respectively, as in their sedentary counterparts [85]. The differences in the animal models as well as in the exercise training protocols (imposing variable flow-mediated shear force) may partially explain the different results. Besides NO pathway, ExT was shown to increase H_2O_2/VEGF/p-Akt axis in the lungs of MCT rats after training [32], suggesting a beneficial role of exercise in angiogenesis and collateral blood flow. However, no change in RV afterload estimated by AT/ET ratio was noted. Figure 17.3 summarizes the main changes modulated by ExT in the lungs.

5 Conclusion

Despite the obvious differences between animal models and exercise training programs, the available pre-clinical data consistently signal a beneficial effect of ExT on RV function in PAH that is mainly dependent on the stage of the disease, exercise intensity and mode. These benefits occurred even in the presence of persistent RV afterload and were associated with the development of an adaptive cardiac phenotype. Regarding the impact of ExT on the lungs, the evidence is very limited and it is not clear if exercise improves pulmonary vascular resistance through NO-mediated pulmonary vasodilatation, modulation of pulmonary artery architecture or both.

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