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Eccentric and concentric cardiac hypertrophy induced by exercise training: microRNAs and molecular determinants

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Abstract

Among the molecular, biochemical and cellular processes that orchestrate the development of the different phenotypes of cardiac hypertrophy in response to physiological stimuli or pathological insults, the specific contribution of exercise training has recently become appreciated. Physiological cardiac hypertrophy involves complex cardiac remodeling that occurs as an adaptive response to static or dynamic chronic exercise, but the stimuli and molecular mechanisms underlying transduction of the hemodynamic overload into myocardial growth are poorly understood. This review summarizes the physiological stimuli that induce concentric and eccentric physiological hypertrophy, and discusses the molecular mechanisms, sarcomeric organization, and signaling pathway involved, also showing that the cardiac markers of pathological hypertrophy (atrial natriuretic factor, β-myosin heavy chain and α-skeletal actin) are not increased. There is no fibrosis and no cardiac dysfunction in eccentric or concentric hypertrophy induced by exercise training. Therefore, the renin-angiotensin system has been implicated as one of the regulatory mechanisms for the control of cardiac function and structure. Here, we show that the angiotensin II type 1 (AT1) receptor is locally activated in pathological and physiological cardiac hypertrophy, although with exercise training it can be stimulated independently of the involvement of angiotensin II. Recently, microRNAs (miRs) have been investigated as a possible therapeutic approach since they regulate the translation of the target mRNAs involved in cardiac hypertrophy; however, miRs in relation to physiological hypertrophy have not been extensively investigated. We summarize here profiling studies that have examined miRs in pathological and physiological cardiac hypertrophy. An understanding of physiological cardiac remodeling may provide a strategy to improve ventricular function in cardiac dysfunction.

Key words: Exercise training; Cardiac hypertrophy; Renin-angiotensin system; AT1 receptor; Akt; MicroRNAs

Physiological cardiac hypertrophy

The term “athlete’s heart” has been widely used to characterize the changes that occur in the heart due to long-term physical exercise in athletes. Physical exercise can be classified as static or dynamic and leads to two different kinds of intermittent chronic cardiac workload, which induces morphological changes in the heart, such as concentric and eccentric physiological cardiac hypertrophy, characterized by a uniform profile of ventricular wall and septum growth (1-3). In the subsequent sections we describe the hemodynamic alterations and the molecular mechanisms responsible for the concentric and eccentric cardiac hypertrophy induced by exercise training. We also describe the involvement of the angiotensin II type I (AT1) receptor and of the microRNAs (miRs) in different forms of cardiac hypertrophy.

Concentric cardiac hypertrophy induced by resistance training

In static or isometric physical exercise (e.g., weight lifting, weight and hammer throwing, wrestling and bodybuilding), strength is developed with little or no movement. This physical exercise, when chronically performed, is known as resistance training, which is a specialized method of conditioning designed to increase muscle strength and power. Both skeletal and cardiac muscles adapt themselves in response to this type of training. Resistance training results in hemodynamic altera-

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tion with marked elevation of blood pressure (BP), leading to pressure overload in the heart, resulting in the parallel addition of sarcomeres. This leads to a predominant increase in cardiomyocyte cell width and consequently to an increase in left ventricular wall thickness without reducing the size of the internal cavity in diastole, with the development of concentric left ventricular hypertrophy (1,2,4). The increase in wall thickness induced by pressure overload is mainly due to an increase in cardiomyocyte cross-sectional area (5) (Figure 1).

In contrast to hypertensive conditions described above, when there is continuous pressure overload, the cardiovascular response to this exercise model is characterized by the intermittent increase in BP during exercise (6,7). An increase of 480/350 (systolic and diastolic) BP has been shown in bodybuilders while performing leg press exercise (6,7). Concentric cardiac hypertrophy might also result from pressure overload observed in many pathologic conditions, such as hypertension (8). However, this type of hypertrophy is followed by diastolic and/or systolic dysfunction and a disproportionate increase in the thickness of the left ventricle posterior wall and interventricular septum (8-10). Sometimes, the physiological hypertrophy developed by high level strength athletes presents a macroscopic structure similar to pathological hypertrophy, which could be incorrectly interpreted as pathological. Moreover, similar adaptations are usually found in athletes who use anabolic steroids associated with resistance training (11). This has also been found when these drugs were associated with aerobic training in experimental animals (12).

Since it is difficult to dissociate the use of metabolic supplements from the abusive use of drugs in athletes who practice resistance training, experimental models can be used for the purpose of study, and one of their major advantages is the ability to precisely control the environmental and food intake conditions of all animals. We have characterized the cardiac adaptation induced by resistance training (13,14) according to a model adapted from Tamaki et al. (15).

The left ventricular mass assessed by echocardiography was 8, 12, and 16% larger in the resistance-trained group than in the control group in the first, second and third months, respectively. This hypertrophy showed a similar increase in the interventricular septum and in the free posterior wall mass. There was no reduction in the end-diastolic left ventricular internal diameter during the 3-month resistance-training period accompanied by maintenance of ventricular function, showing that this stimulus leads to concentric physiological cardiac hypertrophy (13,14).

We have shown that in rats trained at 75% of 1 repetition maximum (1-RM) the left ventricle systolic pressure decreased 13% and there was an increase in the isometric force developed by the papillary muscles of rats. The improvement in cardiomyocyte contractility was due to an increase in myosin ATPase activity and an enhanced Ca^{2+}-influx (16). The double

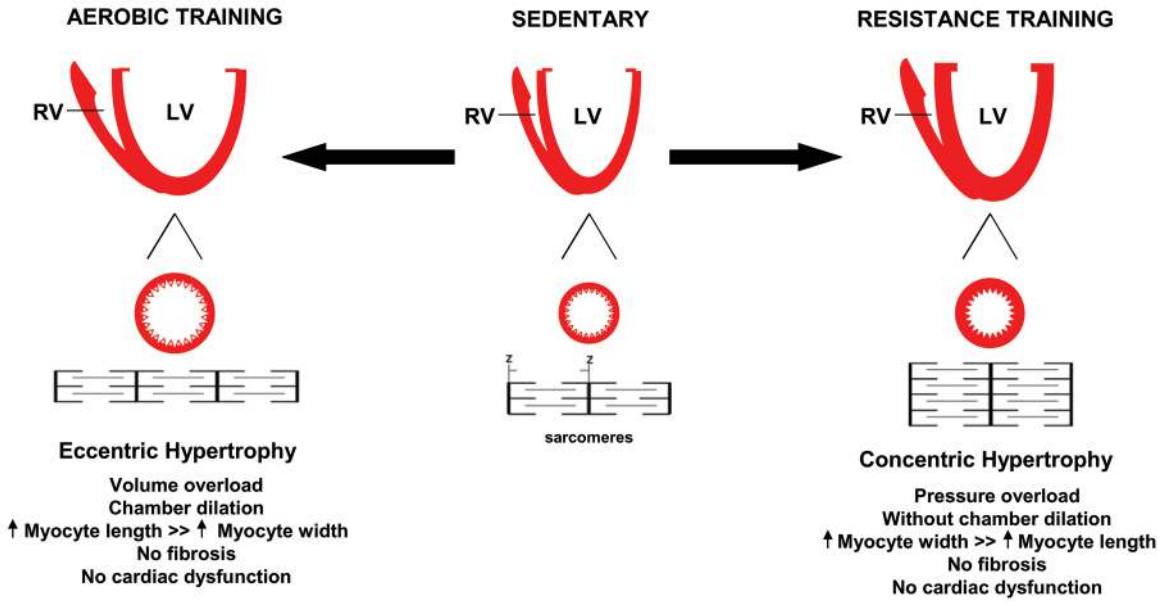

Figure 1. Effect of exercise training on cardiac hypertrophy. Physiological hypertrophy is characterized by a uniform profile of ventricular wall and septum growth, without fibrosis and cardiac dysfunction. Aerobic exercise training, such as long-distance running or swimming, is matched with an increased volume overload accompanied by cardiac chamber dilation, referred to as eccentric hypertrophy. This phenotype is associated with the addition of sarcomeres in series to lengthen the cardiomyocyte and to increase the width of the cell in parallel. In contrast, resistance training, such as weight lifting and wrestling, results in an increased pressure overload without chamber dilation, referred to as concentric hypertrophy. This phenotype is associated with the addition of sarcomeres in parallel to an increase in the cross-sectional cardiac area. LV = left ventricle; RV = right ventricle.

product, an index of cardiac workload, was 18% lower in the trained group after four weeks of resistance training (13). The cardiovascular adaptations observed depended on the training exercise and were not influenced by stress, since circulating catecholamine levels and adrenal gland weights were unchanged (13).

We have shown that none of the pathological cardiac hypertrophy molecular markers, atrial natriuretic factor (ANF) or α-myosin heavy chain (α-MHC)-to-β-MHC ratio, were changed in resistance-trained rats (17). It is well known that cardiomyocyte contractility depends on the expression of α- and β-MHC, since MHC is the major contractile protein of the heart, and is crucial to the efficiency of cardiac performance. The α/β-MHC ratio varies in response to physiological and pathological signaling. Studies have shown a shift from α- toward β-MHC composition of the adult heart under pathological conditions accompanied by higher expression of fetal gene reprogramming, which correlates with impaired cardiac performance (18). Thus, resistance training induces physiological concentric cardiac hypertrophy, and could be used as a good exercise modality to compare physiological and pathological concentric hypertrophy in biochemical, molecular and cellular analyses.

Eccentric cardiac hypertrophy induced by endurance training

In dynamic exercise, in which athletes perform isotonic exercises (e.g., swimming, cycling, and running), the main hemodynamic changes are increased heart rate and stroke volume, the two components of cardiac output. In parallel, an increased effectiveness of the skeletal muscle pump and decrements in peripheral vascular resistance increase the venous return to the heart. Therefore, the heart overload occurs predominantly as a result of the volume, leading to the development of eccentric left ventricular hypertrophy (1-3).

As shown in Figure 1, the eccentric hypertrophy induced by aerobic or endurance training is predominantly characterized by the addition of sarcomeres in series, which leads to an increase in myocyte cell length and consequently increases the cardiac mass with increased chamber volume (1-3).

To study eccentric cardiac hypertrophy, two different aerobic training protocols, both lasting a total of 10 weeks, were performed with rats. The first, a low-intensity, long-duration exercise protocol, consisted of swimming sessions of 60-min duration, 5 days a week, for 10 weeks. The second, also consisting of low-intensity, high-volume training, differed from the first because the animals performed the same swimming training protocol as described above only until the end of the 8th week. In the 9th week, they trained twice a day and the training consisted of swimming sessions of 60-min duration with a 6-h interval between sessions. In the 10th week, they trained three times a day in swimming sessions of 60-min duration with a 4-h interval between sessions. The aim of increasing training frequency (second protocol) was to induce robust cardiac hypertrophy (19). As a result, the left ventricle hypertrophy observed was 13% for the first swimming training protocol and 27% for the second.

In contrast to pathological cardiac hypertrophy (18), the eccentric physiological hypertrophy reported here was not associated with activation of fetal marker genes of pathological cardiac hypertrophy. Our results showed that the first swimming training protocol did not modify the gene expression of ANF, skeletal α-actin or α/β-MHC, whereas the second training protocol significantly reduced the left ventricle levels of skeletal α-actin by 53% and increased the left ventricle levels of α/β-MHC by 98% (20). α-MHC has been associated with increased myosin ATPase activity and enhancement of contractility, corroborating the improvement of ventricular function observed with aerobic training (21).

Chronically performed dynamic exercise or aerobic endurance training induces adjustments in the cardiovascular system (1-3). With regard to the cardiovascular effects, resting bradycardia has been considered to be the hallmark of aerobic exercise training adaptation (12,19,22). The mechanisms underlying the resting bradycardia are strongly dependent on the exercise training mode. Resting bradycardia observed after aerobic training has been explained by a reduction in sympathetic cardiac effects, increased cardiac vagal effects, reduction in intrinsic heart rate, and longer atrioventricular conduction time (22). Medeiros et al. (22) have provided evidence showing that resting bradycardia induced by swimming training is mainly parasympathetically mediated and differs from other dynamic training modes. The development of resting bradycardia indicates that aerobic conditioning was achieved with the aerobic training regimens.

Associated with resting bradycardia, the increase peak oxygen uptake is a well-known adaptation to long-term endurance training. Recently, Steding et al. (3) showed that total heart volume is a strong and independent predictor of peak oxygen uptake or maximal work capacity. Long-term endurance training is associated with a balanced physiological enlargement of the left and right ventricles in both males and females.

In general, in most forms of physical exercise or physical conditioning programs, there is a combination of dynamic and static components. Therefore, the physiological hypertrophy that normally occurs is a combination of different degrees of both concentric and eccentric hypertrophy, leading to mixed cardiac hypertrophy, as observed in triathletes (23). Based on this fact, experimental models can be useful to study the separate effects. Moreover, the degree of physiologic hypertrophy observed is related to the intensity and duration of the exercise sessions, as well as to the type of physical training program, and is directly related to aerobic or resistance capacity. Although cardiac hypertrophy induced by treadmill and swimming training is commonly observed, in some cases these types of training have failed to induce cardiac hypertrophy (24). We have observed that swimming training induces robust cardiac hypertrophy when compared to treadmill training in rats and mice (19,20,22,24,25). In fact, a different cardiac adaptation should be expected after swimming training, since this training

mode differs from running training with respect to body position in the water, water pressure and temperature regulation. The literature shows a different magnitude of cardiac hypertrophy depending on the training protocols (24); however, few studies comparing the effect of different endurance or resistance training modes on cardiac hypertrophy have been conducted in different species.

Exercise-induced cardiac hypertrophy via AT1 receptor

Considerable research efforts have been focused on the molecular mechanisms responsible for transducing hemodynamic load into cardiac growth induced by exercise training. An elevated number of intracellular signaling pathways have been identified as important transducers of the physiological hypertrophic response in cardiomyocytes (2). Several studies have indicated that the local renin-angiotensin system (RAS) is activated by hemodynamic overload and that the AT1 receptor might play a crucial role in the development of cardiac hypertrophy induced by load (26,27).

The RAS has been studied for over a century and its relevance as a cardiovascular regulator has grown steadily. In the classical RAS pathway, angiotensin II (Ang II) binding AT1 and AT2 receptors acts as a potent regulator of fluid volumes, BP and cardiovascular remodeling. Many of the responses to Ang II mediated by the AT1 receptor can be deleterious, such as sympathetic nervous system activation resulting in vasoconstriction and increased heart rate and force of contraction or contractility, as well as promoting cardiac hypertrophy and fibrosis, while the AT2 receptor counteracts the effects of the AT1 receptor, representing a protective mechanism in the heart (26,27).

Under pathological conditions such as hypertension (8), myocardial infarction (9) and heart failure (10), local cardiac RAS levels are increased by augmented protein, angiotensinogen (AGT), angiotensin-converting enzyme (ACE) and Ang II, inducing pathological cardiac hypertrophy and left ventricle dysfunction. In the heart, Ang II has been shown to play a role in the development of cardiac fibrosis via induction of fibroblast proliferation and collagen disposition (26-29). In addition, Ang II, as a growth factor, may contribute to the development of smooth muscle and cardiac cell hypertrophy (26-29). In fact, Sadoshima et al. (28) showed that the mechanical stretch *in vitro* causes acute release of Ang II from cardiomyocytes within 10 min, and the expression of the AGT gene after 6 h. They also showed that Ang II acts as an initial mediator of the stretch-induced hypertrophic response. The role and mechanisms of Ang II via the AT1 receptor in cardiac hypertrophy have been associated with increased expression of many immediate-early genes (c-fos, c-jun, junB, Egr-1, and c-myc) and fetal marker genes of cardiac hypertrophy (ANF, skeletal α-actin, β-MHC) conferring a pathological phenotype (29).

In spite of many studies, it is still unclear whether activation of the cardiac RAS in response to hemodynamic overload predominantly occurs in cardiomyocytes or fibroblasts or both. Recent evidence has indicated that cardiac Ang II levels are implicated in the induction of fibrosis, but Ang II is not required for left ventricle hypertrophy (30). Using transgenic animal models for RAS components it has been shown that accentuated formation of local Ang II in the heart (20- to 50-fold greater than the levels seen in control groups) was not responsible for the development of the hypertrophy observed. In the same study, using another type of transgenic mouse that overexpressed a degradation-resistant form of Ang II, the hormone levels reached 100-fold the normal levels and began to spill into the circulation. Although an increase in fibrosis was shown, hypertrophy continued only when an excess amount of cardiac Ang II entered the circulation and caused an increase in BP (30,31). More recently, Xiao et al. (32) reported that in mice expressing ACE only in the heart the increase in cardiac Ang II was not associated with cardiac hypertrophy, indicating that the increase of cardiac Ang II was not sufficient to induce hypertrophy. In addition, transgenic mice harboring one, two, three, or four copies of the ACE gene showed that the magnitude of physiological cardiac hypertrophy induced by swimming training was not associated with different ACE levels (25). Taken together, these results suggest that cardiac hypertrophy induced by Ang II depends on an increase in BP.

In contrast, several studies have implied that animal models overexpressing the AT1 receptor demonstrated the induction of cardiac hypertrophy (33). Zou et al. (34) showed *in vitro* and *in vivo* that the AT1 receptor is a mechanical sensor and that it converts mechanical stress into a biochemical signal inducing left ventricle hypertrophy without the involvement of Ang II. In agreement, Yasuda et al. (35) showed conformation changes in the AT1 receptor when activated by mechanical stress independently of Ang II, through the anticlockwise rotation of transmembrane 7 domains, translating it into biochemical signals inside the cardiac cell. Thus, these distinct responses of the RAS components to hypertrophic stimuli show that the RAS is an important modulator of cardiac function and structure.

Large clinical trials evaluating the blockade of RAS with ACE inhibitors or angiotensin receptor blockers have demonstrated an ability to prevent progression and to induce regression of cardiac hypertrophy improving ventricular performance (36,37). Indeed, the chronic administration of an AT1 receptor antagonist (Losartan) resulted in the reversal of fibrosis, inhibition of the post-transcriptional synthesis of procollagen type I, inhibition of tissue inhibitor of metalloproteinase-1 expression and stimulation of collagenase activity in the left ventricle of spontaneously hypertensive adult rats (38), thereby reducing the significant and independent cardiovascular risk conferred by cardiac remodeling.

In the last decade, investigations have pointed out a cardioprotective role played by the novel branch of the RAS. The recently discovered member of the RAS, ACE2, is an essential regulator of heart function (38) and has been used as a negative indicator of cardiovascular disease (CVD) due to its pivotal role in Ang (1-7) formation, implicated in several

biological effects that are opposite to those elicited by Ang II, acting in anti-proliferation and vasodilator actions and anti-fibrosis effects (39,40). Higher cardiac Ang II levels were associated with genetic deletion of ACE2 in mice and resulted in the development of severe cardiac dysfunction (38). Furthermore, Ang (1-7) can reduce hypertension-induced cardiac remodeling through a direct effect on the heart (39). In addition to the known effect of AT1 receptor blockade in hindering the action of Ang II, this intervention stimulates the cardiac AT2 receptor and accelerates the processing of Ang II by the action of ACE2, inducing vasodilator and anti-fibrotic effects and conferring therapeutic benefits on patients with CVD (40). Moreover, the continuous administration of Losartan increased cardiac ACE2, mRNA expression and activity, and increased cardiac Ang (1-7) levels. Previous findings have suggested that ACE2 maintains the important balance between Ang II and Ang (1-7) levels, favoring cardiovascular homeostasis (40).

Studies have shown that exercise training attenuates deleterious cardiac remodeling and also preserves cardiac function in CVD, and that these beneficial effects may be due in part to the exercise training-induced attenuation of the RAS (10). A recent study demonstrated that aerobic exercise training decreased cardiac Ang II levels and ACE activity in a genetic model of sympathetic hyperactivity-induced heart failure, while it increased ACE2 expression and prevented exercise intolerance and ventricular dysfunction with little impact on cardiac remodeling (10). Taken together, these data provide evidence that reduced cardiac RAS explains at least in part the beneficial effects of exercise training on cardiac function. There are only limited data about the effects of exercise training, either associated with CVD or not, on cardiac RAS regulating cardiac hypertrophy.

Interestingly, we recently showed that AT1 receptor blockade prevents physiological left ventricle hypertrophy induced by resistance training (17) and aerobic exercise training (19,41). These studies underscore the importance of a better understanding of aerobic and resistance training and the regulation of left ventricle hypertrophy by the RAS (Figure 2).

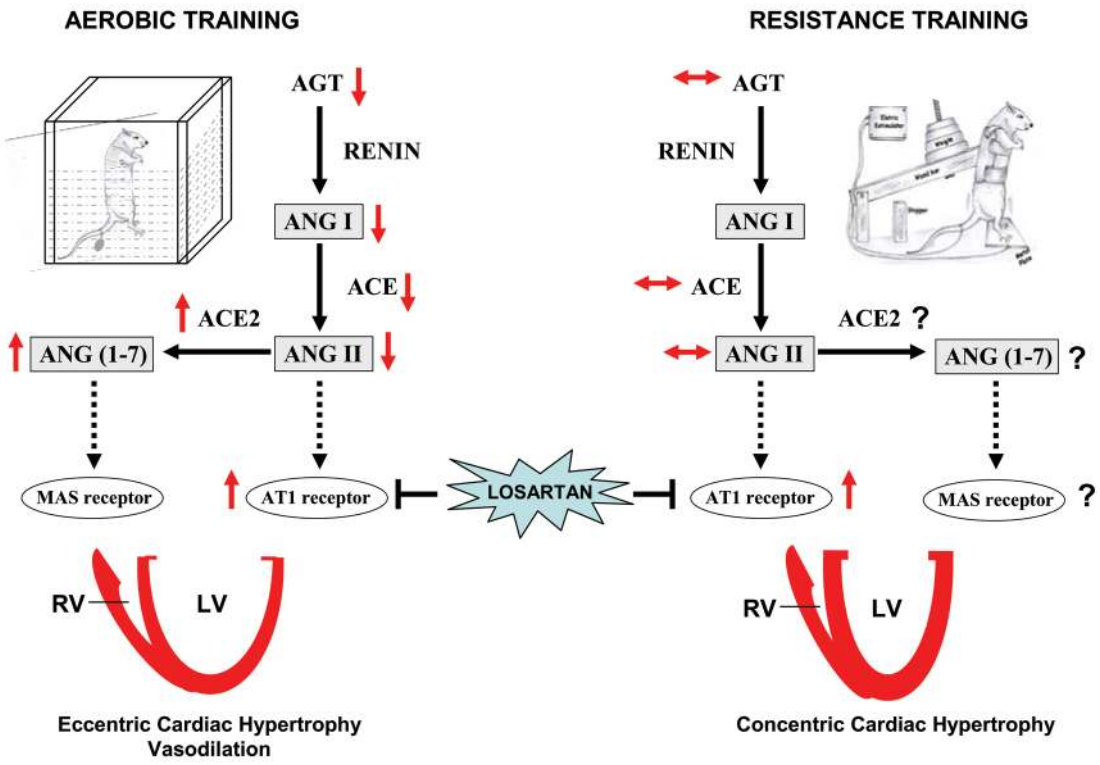

Figure 2. Schematic presentation of the effects of exercise training on the cardiac renin-angiotensin system (RAS). Aerobic and resistance training induced eccentric and concentric cardiac hypertrophy, respectively, via the angiotensin II type 1 (AT1) receptor, and both types of physiological cardiac hypertrophy were prevented by AT1 receptor blockade (Losartan). We suggest that aerobic training shifts the generation of angiotensin peptides away from angiotensin II (ANG II) and towards ANG (1-7), implicated in vasodilation and anti-fibrosis. In contrast, resistance training did not modify these cardiac RAS components. Red arrows indicate points of up-regulation, down-regulation or maintenance of RAS components induced by exercise training. ACE = angiotensin-converting enzyme; ACE2 = angiotensin-converting enzyme 2; ANG (1-7) = angiotensin (1-7); AGT = angiotensinogen; RV = right ventricle; LV = left ventricle.

Our first study (17) focused on the role of the RAS in cardiac hypertrophy induced by resistance training in rats. We observed that AT1 receptor expression increases in response to training without any alterations in the other components of the RAS. Furthermore, treatment with the AT1 receptor blocker Losartan prevented this adaptive hypertrophy (Figure 2). Indeed, our results showed that physiological hypertrophy is locally regulated and is independent of the systemic RAS. The implications of these results are 2-fold: first, AT1 receptor stimulation is necessary for the development of cardiac hypertrophy in response to resistance training even in the absence of increased Ang II; second, the mechanical stretch due to resistance training increases AT1 receptor expression. Other cardiovascular adaptations in this animal model have already been described by us (13,14), as previously documented above.

Nevertheless, the role of the RAS in non-pressure overload hypertrophy models, such as aerobic exercise training, has not been extensively investigated. Therefore, the first study demonstrating the hypertrophic response of the adult rat heart to chronic conditioning in the presence and absence of an AT1 receptor antagonist showed that AT1 receptor blockade did not prevent physiological cardiac hypertrophy induced by a chronic swimming program (42). However, the authors treated the animals with an AT1 receptor only twice a week, probably reducing the necessary potential inhibition for cardiac hypertrophy blockade. In addition, another potential criticism of this study is that exercise-associated adaptations that reflect integrative cardiovascular physiology, such as an increase in peak oxygen consumption and a decrease in resting heart rate, or those that may occur in skeletal muscle, such as increases in muscle succinate dehydrogenase and citrate synthase activities, were not determined (42).

In contrast, our results with swimming training have shown that the treatment with Losartan prevented left ventricle hypertrophy (Figure 2). Similar to resistance training, cardiac hypertrophy was not decreased when the systemic RAS was inhibited by chronic salt treatment, which suggests that the left ventricle hypertrophy induced by swimming training depends on the local RAS activity instead of the systemic RAS (19). This was the first report of a detailed study performed to investigate the participation of the local versus systemic RAS in cardiac hypertrophy induced by aerobic exercise training, since all the markers of aerobic exercise training were confirmed with these protocols.

Interestingly, in a recent study (41) we showed that cardiac AGT and Ang I levels, ACE activity and protein expression, as well as Ang II levels were lower in swimming-trained rats, although AT1 receptor levels increased after training. In addition, ACE2 and Ang (1-7) levels were increased in the hearts of swimming-trained rats. Therefore, we suggested that there was an increased exercise-induced cardiac AT1 receptor without Ang II participating in the cardiac hypertrophy and that the discovery that ACE2 and Ang (1-7) are increased in the heart by exercise suggests that this non-classic cardiac RAS counteracts the classic cardiac RAS. The counterbalancing effect increases vasodilatation and cardiac hypertrophy in response to the demands of aerobic capacity for greater transport of blood and oxygen supply to the exercising cardiac muscle (41) (Figure 2). Thus, our results suggest that AT1 receptor activation, but not Ang II, may participate in physiological cardiac hypertrophy induced by both resistance and aerobic types of exercise training.

The molecular signaling pathways linking aerobic and resistance training to physiological cardiac hypertrophy have not been well established. Zhai et al. (43) and other investigators have defined a novel signaling pathway of mechanical stretch, demonstrating the activation of protein kinase B (PKB or Akt) by transactivation of epidermal growth factor receptor (EGFR) via the AT1 receptor. Recent studies have shown that Akt activation is a critical determinant of the intracellular signaling pathway in the physiological growth of the heart induced by aerobic and resistance training (44,45). DeBosch et al. (44) observed impaired protein synthesis in Akt1 knockout ($Akt1^{-/-}$) mouse cardiomyocytes after insulin-like growth factor 1 (IGF-1) stimulation. In addition, the authors showed that cardiac hypertrophy assessed by measurement of the left ventricle weight/tibial length ratio, cardiomyocyte cross-sectional area, and cardiac function assessed by transthoracic echocardiography were significantly impaired in $Akt1^{-/-}$ swimming-trained mice in 90-min sessions twice daily for 20 days when compared with congenic sedentary controls, indicating that Akt1 is required for physiological cardiac hypertrophy (44). In contrast, pathological cardiac hypertrophy did not activate the Akt signal cascade and in some cases the absence of Akt1 profoundly exacerbated the transverse aortic constriction or endothelin 1-stimulated pathological cardiac hypertrophic, suggesting that the Akt activation pathway may differentiate between physiological and pathological hypertrophy (44,45).

Accordingly, we observed that the swimming training protocols increased the cardiac phosphoSer_{473}-Akt protein expression, indicating its participation in physiological cardiac hypertrophy (20). Therefore, in a recent study we showed that a session of strength exercise consisting of 4 sets of 12 repetitions with an 80% work overload promoted increased Akt activity in a group exercised and killed 30 min after the strength training session. This increased Akt activity was also accompanied by an increase in phosphorylation of mammalian target of rapamycin (mTOR). Interestingly, these effects were blocked by Losartan treatment in the exercised groups, suggesting activation of the AT1 receptor-Akt-mTOR signaling pathway after the resistance exercise session - a potential mechanism for cardiac hypertrophy (46).

Although a large number of studies have shown that

Akt is cardioprotective, others using transgenic mice with cardiac-specific expression of activated Akt have demonstrated that chronic Akt activation has detrimental effects after ischemia/reperfusion injury, due to feedback inhibition of phosphoinositide 3-kinase (PI3K) activity through both proteasome-dependent degradation of insulin receptor substrate-1 (IRS-1) and inhibition of transcription of IRS-1 and IRS-2. This mechanistic connection is demonstrated by the rescue of function and reduced injury seen after restoration of PI3K signaling. Thus, the authors demonstrated that PI3K-dependent but Akt-independent pathways are crucial for full cardioprotection and suggest a mechanism by which chronic Akt activation may become maladaptive (47). A possible explanation for the apparent controversial results of the literature could be the origin of Akt activation, since the models being considered are undoubtedly different, one being an exercise training model and the other represented by transgenic mice with exacerbated Akt activation. This would imply, at least in part, distinct effects of Akt-driven cardiac hypertrophy signaling since exercise-induced stress would be an intermittent stimulus, while it would be continuous and in transgenic mice.

Among the molecular processes that control cardiac hypertrophy in response to physiological or pathological stimuli, the contribution of miRs has recently become appreciated. Recent studies have shown the development of miR-regulated pathological cardiac hypertrophy (48). In addition, miRs may be important for normal development and for physiological cardiac hypertrophy induced by exercise training, at least in part, regulating target genes that contribute to physiological left ventricle hypertrophy, such as the cardiac RAS genes.

MicroRNAs can be tiny modulators of cardiac hypertrophy induced by exercise training

MiRs are short, single-stranded RNAs that have few nucleotides (17-22), and work as “brakes” for many cellular processes, since they regulate the protein expression of target genes. The mechanism of action of an miR is called post-transcriptional, i.e., these molecules are coupled with partial complementarity in regions 3’ untranslated mRNA targets, inhibiting the translation of the mRNA into a protein. Several miR genes cluster into families based on their sequence similarity, especially to the region covering nucleotides 2-8 of the 5’ portion called the “seed region”, in which the base pairing between the miR and its mRNA is perfectly complementary. It is considered that miRs with identical seed regions may target the same sets of genes (48).

The biogenesis of miRs is accomplished through sequential enzymatic reactions: RNA polymerase II transcribes primary miR (pri-miR) that receives the action of two RNase III endonucleases: the first called DROSHA, that cleaves pri-miR into pre-miR, and the second in the cytoplasm, called DICER, that generates a duplex containing mature miRs, ready to exert the suppression of translation initiation, where the RNA-induced silencing complex (RISC) component prevents the binding of eIF-4E, a translation factor in the 7-methylguanosine cap region of a targeted mRNA (49).

Several *in vivo* and *in vitro* studies have shown that miRs regulate a myriad of cellular processes, including heart development and cardiac hypertrophy, both under physiological and pathological conditions (50-53). Some important issues have been highlighted regarding the participation of miR in the process of pathological cardiac hypertrophy, since many targets can be regulated simultaneously or synergistically by one or more miRs, and miRs also can be regulated by molecular bases involved in cardiac hypertrophy and other processes, reinforcing or attenuating its phenotype (53-55). A recent review outlined some functional concepts about the action of miRs on their targets, which may be intronic for multiple targets, by functional cooperativity as well as physiological buffering (56).

Evidence has shown a signature pattern of miRs that are up-regulated or down-regulated in pathological cardiac hypertrophy: miR-1, miR-29, miR-30, miR-133, and miR-150 frequently are down-regulated, while miR-21, miR-23a, miR-125, miR-195, miR-199, and miR-214 are up-regulated. Nevertheless, there has been little investigation about miRs and physiological cardiac hypertrophy (51,57).

The miR family more firmly established as a regulator of cardiac hypertrophy, and which is highly expressed in the heart, undoubtedly comprises the miR-1, 133a and 133b family. The *in vitro* overexpression of miR-133 and miR-1 inhibited cardiac hypertrophy (50). In contrast, suppression of this miR-133 induced pronounced cardiac hypertrophy. Additionally, the cited study showed that both miR-1 and miR-133 were down-regulated in three models of cardiac hypertrophy: transverse aortic constriction, Akt transgenic mice and high-intensity interval training and physiological concentric cardiac hypertrophy. The main target genes that were validated were the Ras homologue gene family-A (Rhoa), cell division control protein 42 (Cdc42) and Wolf-Hirschhorn syndrome candidate 2 (NELFA protein/WHSC2), which play a role in a general program of hypertrophy and are valid both for physiological and pathological cardiac hypertrophy (50).

We have investigated whether the physiological eccentric cardiac hypertrophy induced by two different swimming training protocols described above could induce a similar expression profile for these miRs. We showed that the expression of miR-1, 133a and 133b was similar to the expression in pathological and concentric cardiac hypertrophy. Additionally, miR-1, 133a and 133b are similarly down-regulated in both aerobically trained groups related

to sedentary animals, also showing a common pattern of expression that suggests an involvement in general programs for cardiac hypertrophy (Figure 3) (20). However, there is an unanswered question about whether other miRs are differentially expressed in physiological cardiac hypertrophy when compared to the pathological type and thus further investigation is necessary to delineate and differentiate the expression of miRs in concentric and eccentric cardiac physiological hypertrophy (Figure 4).

Another *in vitro* study with isoproterenol and aldosterone treatment showed that miR-23 also is a pro-hypertrophic miR, and that under pressure overload its expression was shown to be up-regulated by the transcription nuclear factor of activated T-cells 3 (NFATc3) that directly activates miR-23a expression through the transcriptional machinery. In turn, muscle ring-finger protein-1 (MuRF1) is a target of miR-23, since its protein level and MuRF1-3'UTR were both suppressed in a dose-dependent manner with constructs of miR-23a. Knockdown of miR-23a could attenuate cardiac hypertrophy, suggesting that miR-23a is able to convey the hypertrophic signal (52).

Moreover, the overexpression of miR-195, considered a stress-inducible miR, in transgenic mice resulted in dramatic pathological cardiac remodeling and heart failure, showing that miR-195 alone was sufficient to drive cardiac hypertrophy. MiR-195 initially induced cardiac growth that progressed to a dilated phenotype (57).

In a study using mice and surgery with aortic banding, miR-21 was increased by more than 4-fold when compared with the sham surgical group. Similar aberrant expression of the most up-regulated miR, miR-21, was also found in cultured neonatal hypertrophic cardiomyocytes stimulated with Ang II or phenylephrine. Modulation of miR-21 expression via antisense-mediated depletion (knockdown) had a significant negative effect on cardiac hypertrophy and up-regulation of miR-21 expression-induced cardiac hypertrophy. The targets that have already been validated are mitogen-activated protein kinase (MAPK), FasLigand (FasL), phosphatase tensin homolog deleted on chromosome 10 (PTEN), which stimulate the Akt pathway. Recently, antagonist results were observed in miR experiments on animals. Reports have suggested that an miR-21 antagomir might be therapeutically useful in preventing heart failure in mice, although genetic deletion of miR-21 did not confer a dysfunctional phenotype, suggesting possible confounding factors that may be a product of technical variations, miR redundancy or nonspecific effects of a cholesterol-modified antagomir (54,55).

The miR-29 family has also been validated as a regulator of gene expression of collagen I, collagen III and fibrillin I, and is involved in pathological cardiac hypertrophy and in heart failure processes. The down-regulation of miR-29 *in vitro* and *in vivo* using a mouse myocardial infarction model enhanced the fibrotic response and expression of collagens, whereas overexpression of miR-29 in fibroblasts reduced

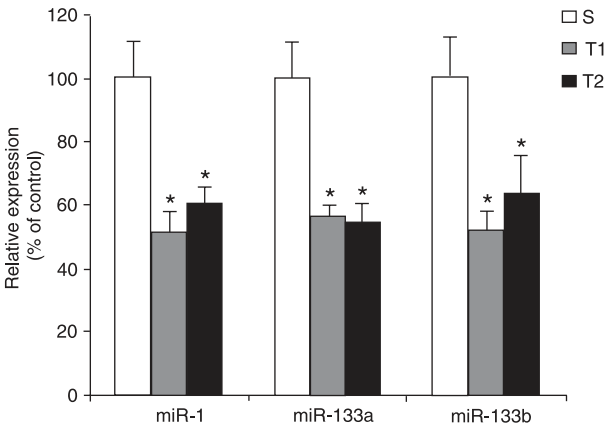

Figure 3. Effect of exercise training on relative expression of cardiac miR-1, miR-133a and miR-133b analyzed by real-time PCR. miR = microRNA; S = sedentary Wistar rats; T1 = rats trained by swimming sessions for 10 weeks, 60 min per session, 5 days per week; T2 = similar to T1 until the end of the 8th week; in the 9th week rats swam two sessions per day and in the 10th week they swam three sessions per day. *P < 0.01 compared to S rats (one-way ANOVA and Tukey *post hoc* test). N = 5 animals/group. Data are reported as means ± SD. miR = microRNA.

collagen expression (53). Recently, we showed that rats subjected to two swimming training protocols with moderate- and high-volume training presented physiological cardiac hypertrophy and increased gene expression of miR-29, which were linear with the increase in training volume. The miR-29 up-regulation was accompanied by improvement in ventricular compliance and was closely correlated with the decrease in gene and protein expression of collagens I and III. These data suggest that increased expression of miR-29 plays an important protective role in the control of cardiac collagen and hypertrophy induced by aerobic training (Figure 4) (20).

The miR-208 is called "MyomiR" due to the fact that it is one among others (miR-499 and miR-206) exclusively expressed in muscle tissues. MiR-208 is expressed by intron 27 of the α-MHC and is in the top hierarchy of regulatory steps since it controls Myh7b or β-MHC gene expression. MiR-208 targets several genes that are transcriptional repressors of the β-MHC gene and also activates slow fiber gene expression. These targets were validated by comparison of the expression of corresponding proteins in heart extracts from wild and knockout mice for miR-208, and by the generation of transgenic mice that overexpress this miR and extinguish β-MHC gene expression or protein, suggesting that this miR leads to a network of miRs that regulate the pattern of fiber and muscle performance (58).

Although the RAS plays a critical role in regulating physiological and pathological processes in the cardiovascular system, very few of the predicted sites have been experimentally validated. The mouse miR-143/145 cluster,

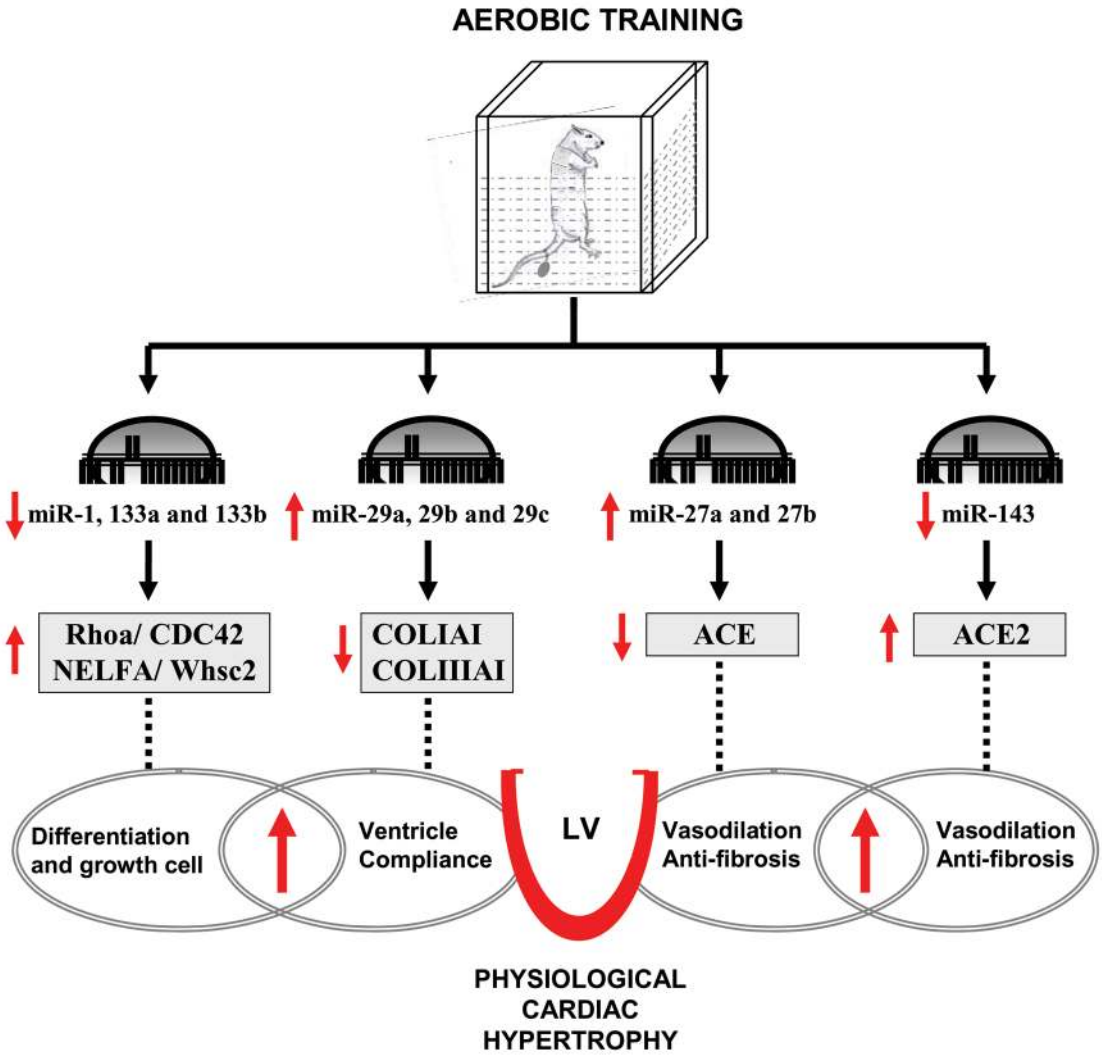

Figure 4. The regulation of miRs by aerobic training controls phenotypes that contribute to physiological cardiac hypertrophy. Aerobic exercise training is a powerful stimulus modulating several miRs that regulate their target mRNAs, thereby contributing to the phenotype of physiological cardiac hypertrophy through different signaling pathways. Red arrows indicate points of up-regulation or down-regulation of miRs, proteins and cardiac function induced by exercise training. Rhoa = Ras homologue gene family-A; CDC42 = cell division control protein 42; NELFA protein/WHSC2 = Wolf-Hirschhorn syndrome candidate 2; COLI and COLIII = collagens I and III; ACE and ACE 2 = angiotensin-converting enzymes 1 and 2; miR = microRNA.

that has exclusive expression in heart and smooth muscle cells during development, is required for acquisition of the contractile phenotype by vascular smooth muscle cells (VSMCs) and plays a role in the regulation of BP. VSMCs from miR-143/145 knockout animals presented incapacitated contractile abilities, favored neointimal lesion development, and protein overexpression of membrane-bound ACE. Pharmacological inhibition of either ACE or the AT1 receptor partially reversed vascular dysfunction and normalized gene expression in miR-143/145-deficient mice. These results may offer a new approach to vascular repair and to the attenuation of arteriosclerotic pathogenesis (59).

Another study has also shown that miR-155 specifically targets a predicted site of the AT1 receptor. Experiments involving transfection of cells that mimic mature miR-155 showed that this miR inhibited the expression of human AT1 receptor and also attenuated Ang II-induced signaling via the AT1 receptor in fibroblasts and VSMCs. Other experiments with antimir have demonstrated that transfection of anti-miR-155 increased AT1 receptor expression and also

enhanced Ang II signaling via this receptor (60).

Despite the fact that the literature has presented studies discussing the relationship of miRs and cardiac hypertrophy in cardiovascular disease and phenotypes, it is important to emphasize that, to our knowledge, only one study involving cardiac hypertrophy induced by physical training and miRs has been published, and the differential expression between the types of cardiac hypertrophy (physiological *vs* pathological) has not been explored. The training protocol adopted was high-intensity training that promoted concentric cardiac hypertrophy, whereas aerobic training promotes eccentric cardiac hypertrophy (2,50). We showed the role of the miRs and their relation with RAS in physiological left ventricular hypertrophy showing biochemical and molecular mechanisms. The exercise training decreased miR-143 that could up-regulate cardioprotective genes such as ACE2, and also increased miRNA-27 that could down-regulate cardiac ACE expression. Together these effects might provide the additional aerobic capacity required by the exercised heart (41).

Since exercise training has beneficial effects on the cardiovascular system, the investigation of the miRs differentially expressed in concentric and eccentric cardiac hypertrophy induced by exercise training can contribute to selecting miRs and establishing a strategy for silencing or overexpressing specific miRs in the therapeutic treatment of cardiac diseases.

In conclusion, most studies in the literature, comparing pathological to physiological cardiac hypertrophy, refer to hypertrophy induced by aerobic physical training. However, it is relevant to show that the concentric cardiac hypertrophy induced by resistance training is also physiological. Both concentric and eccentric cardiac hypertrophy can be induced by physiological (intermittent) and pathological cardiac overload. The patterns of gene and protein expression are induced by different cardiac stimuli or stresses, promoting different forms of cardiac hypertrophy and signaling transduction pathways. The recent class of RNA discovered, miRs, may help to understand the molecular mechanisms responsible for mediating the different forms of cardiac hypertrophy. Since exercise training promotes benefits to the cardiovascular system as a whole, delineating the molecular mechanisms involved in concentric and eccentric cardiac hypertrophy promoted by exercise training may contribute to the development of new therapeutic and non-pharmacological treatment to improve ventricular function in pathological conditions.

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