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Cardiac adaptations to exercise: the role of stem cells and future therapeutic insights

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Abstract

The heart responds to physical exercise through changes in its morphology known as physiological hypertrophy. Besides cardiomyocyte hypertrophy, there is also an increased cellular proliferation with formation of new myocytes. Altogether, these changes lead to an improvement in cardiac function. The molecular mechanism responsible for the development of physiological hypertrophy is centred in the IGF-1R/PI3K/Akt signalling pathway, which modulates the activity of transcription factors involved in protein synthesis and cardiomyocytes proliferation. The endogenous pool of cardiac stem cells is believed to be crucial for the continuous renewal and regenerative potential of cardiac tissue, and has also been positively correlated with physiological hypertrophy. Another system involved in myocardium physiology and pathology is microRNA. The knowledge of the mechanisms underlying the beneficial effects of physical exercise over cardiac function may sustain the development of new therapeutic approaches regarding cardiac pathologies, which represent today an important cause of morbidity and mortality worldwide.

Keywords: Exercise, Heart, Hypertrophy, microRNA, Stem cells.

Introduction

The understanding of heart's physiology has always been on the top of scientific community's priorities. Indeed, there is an enormous amount of work available regarding this area of knowledge.

The heart responds to increased workload with hypertrophy. According to the nature of the stimulus, the hypertrophy can be either physiological or pathological[1]. The main stimulus that leads to pathological hypertrophy is pressure overload, where the heart responds with hypertrophy of existing myocytes as an attempt to overwhelm the increased load[2]. However, in this setting maladaptive changes, such as apoptosis, necrosis and a shift in gene expression, also occur, which leads to rapid decline in cardiac function, with heart failure as possible outcome[1, 2]. In the opposite side there is the physiological hypertrophy, with physical exercise appearing as the best example of this type of cardiac adaptation. Unlike pressure overload, exercise training increases the blood flow through the heart (volume overload)[1], which in turn results in lengthening of myocytes and consequent eccentric hypertrophy of the whole organ[2]. The normal structure of the myocardium is kept, the cardiac function remains unchanged or may improve[3], and the loss of myocytes and increased fibrosis observed in pathological hypertrophy are actually attenuated with exercise training[4].

As noted above, physical exercise is the main trigger of physiological hypertrophy, with all the resulting benefits. Exercise training is today extensively prescribed and is a cheap and effective way for both the prevention and management of heart diseases[3]. Indeed, current evidence points that an improved fitness level is a strong indicator of freedom from all-cause mortality[5] and is enough to decrease the morbidity and mortality linked with cardiac disorders[6]. Besides, physical exercise has been shown to protect the myocardium from age induced apoptosis, fibrosis and senescence[4, 7, 8].

Until the past decade, it was believed that the heart was a post-mitotic organ, with no regenerative potential. However, an increasing body of evidence supports the existence of a resident population of cardiac stem cells[9-12]. This endogenous pool not only reassures the continuous renewal of cardiac tissue[13], but also supports some grade of regeneration after an injury[9, 11]. The old paradigm of the heart as a terminally differentiated organ without any renewal potential did not give space for the exploration of regenerative medicine. However, by the light of the evidence accumulated during the past decade some work begun to be done in the field of regenerative medicine applied to the heart.

This review aims to look over the mechanisms known to take part in the control of the adaptations of myocardium to physical exercise, and open new perspectives about possible applications of this knowledge in the search of new therapeutic approaches.

Macroscopic and functional changes

In 1975 Morganroth *et al.* used echocardiography to describe for the first time the cardiac changes induced by physical exercise, concluding that endurance exercise induced an increase in left ventricle mass and diastolic cavity size, while the wall thickness remained normal (eccentric hypertrophy). On the other hand, athletes that went through a resistance training program showed increased myocardial mass with ventricular wall thickening, but with normal diastolic cavity size (concentric hypertrophy)[14]. This concept has been accepted for decades as the "Morganroth hypothesis". Indeed, other studies have found an increase in both left ventricular mass and left ventricle end diastolic diameter with endurance exercise[15-17], though some of them have failed to show any effect of resistance training in cardiac morphology[15, 16].

Adaptations at cellular level

Unlike the macroscopic remodelling described in the previous paragraph, due to ethical limitations there is a paucity of studies describing the changes that occur at the cellular level in humans. However, a vast amount of studies using mice have shown that an increase in individual cardiomyocyte dimensions occurs in response to several swimming training programmes[18-20]. That increase comprises both the cardiomyocyte's long and short axis and mean cell area[21], and is remarkably related with the exercise's intensity – high intensity exercise induces bigger changes than moderate intensity[6]. On the other hand, the pattern of cardiomyocyte growth is also determined by the type of physical exercise: while endurance training (swimming, running), and the associated volume overload, prompt myocyte lengthening, resistance training (wrestling, weight lifting), which is associated with an intermittent pressure overload, drives mainly to thickening of myocytes[2].

Until recently, cardiomyocyte hypertrophy was thought to be the only mean by which the heart increased its size, which was in line with the paradigm that heart was a post-mitotic organ without any renewal potential. However, nowadays' current evidence indicates that human myocardium is capable of, at least in part, renovate itself by new cardiomyocyte formation[13]. Actually, if it was not this self-renewing capacity, the heart would lose most of its mass in few decades[11]. Furthermore, increased levels of division markers, along with a rise in the number of cardiomyocytes, has already been documented in mice following the application of endurance exercise protocols[18, 22]. Interestingly, the increase in the number of cardiomyocytes was positively related with the intensity of physical exercise the rats have been exposed to[22]. Therefore, it seems that the regenerative capacity of myocardium is enhanced by physical activity.

Another possible mechanism by which the heart can increase its mass is through a drop in apoptotic rate. Indeed, several studies point in this direction. Endurance exercise has been found to attenuate the rise in Bax/Bcl-2 ratio seen with ageing. Consequently there is a decrease in the activation of caspase-9 and 3 and DNA fragmentation, which is reflected in the superior number of myocytes per area observed in old exercised rats compared with sedentary ones[4]. On the other hand, it was also demonstrated a rise in the levels of heat shock protein 70 (HSP70), known to inhibit apoptosis, in the ventricles of exercised mice[7]. At last but not the least, an improved telomerase activity was observed in mice exposed to endurance exercise, thereby protecting myocytes from senescence and apoptosis. This was found already after 21 days of exercise, even before physiological hypertrophy has been documented[8]. Furthermore, in studies using obese rats, endurance exercise was also shown to decrease the number of apoptotic cardiomyocytes[23, 24] and reverse the architectural changes observed to occur in association with obesity[23].

Molecular mechanisms

***IGF-1R/PI3K/Akt pathway*.** The IGF-1R/PI3K/Akt pathway has been extensively studied and is seen as the hallmark of physiological hypertrophy[20, 25, 26]. In fact, the nature of myocardial adaptation to increased workload (physiological vs. pathological) begins to differentiate already at the growth factor and receptor level – while the activation of G-protein coupled receptors by adrenergic stimuli results in pathological adaptation of myocardium[27], in the presence of physiological stimuli such as physical exercise it is the insulin-like growth factor I (IGF-1) acting over its receptor (IGF-1R) and triggering the beneficial adaptations described above[25-27].

In one study using rats, it was shown that swimming training increased the IGF-1 mRNA after 2 and 6 weeks of exercise[28]. These findings are compatible with the work of Serneri *et al*. that accessed the concentration of this growth factor in coronary sinus and concluded that it was significantly bigger in athletes than in controls[29]. The importance of the pair IGF-1/IGF-1R was further highlighted by knock-out studies in which the deletion of IGF-1R in mice attenuated the cardiac hypertrophy seen in controls after swimming training[20]. On the other hand, the overexpression of IGF-1R was associated with a proportional increase in the chamber size and wall thickness of heart, without any histologic signs of cardiomyopathy, what was also reflected in the enhanced systolic function of transgenic IGF-1R animals[25].

IGF-1R is a tyrosine kinase receptor which once stimulated by its ligand phosphorylates and activates phosphatidylinositol 3-kinase (PI3K)[30]. PI3Ks are a family of ubiquitous expressed lipid kinases, which are essential for a wide range of biological processes in several different types of tissues[27]. Class-I PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate to generate phosphatidylinositol-3,4,5-trisphosphate, which in turn acts as second messenger to activate Akt. Class-I_A PI3K is activated by growth factor receptors, such as IGF-1R, while class-I_B PI3K is activated by G-protein coupled receptors[27]. The importance of class-I_A PI3K activity for the development of physiological hypertrophy was well documented by studies in which mice lacking either the catalytic or regulatory subunit of this kinase failed to show physiological hypertrophy[25-27]. However, the same didn't happen when in the presence of a pathological stimulus, since even in the absence of the catalytic subunit of class-I_A PI3K, pressure overload successfully induced pathological hypertrophy[26].

The downstream effector of the IGF-1R/PI3K/Akt pathway is the serine-threonine kinase Akt, also known as protein kinase B (PKB). There have been identified 3

isoforms of this kinase (Akt1, 2 and 3), and despite their high degree of homology, their expression varies among different tissues[31]. Data suggests that the most important isoform in cardiac physiology is Akt1[19, 31]. As noted above, PI3K plays a key role in the activation of Akt through generation of phosphatidylinositol-3,4,5-trisphosphate. PTEN has the opposite effect, and thus decreases the activation of Akt[31]. Akt phosphorylates several different targets, which implicates it in a variety of biological processes[31]. The importance of Akt for the development of physiological hypertrophy is well supported by a study in which mice lacking Akt1 failed in achieving the degree of cardiac adaptation seen in their control littermates after a program of swimming training[19]. In addition, this importance is further reinforced by the evidence that overexpression of Akt causes cardiac hypertrophy both at the molecular and histological level, without any parallel increase in the collagen content of the heart.

Downstream targets. The mammalian target of rapamycin (mTOR) works as an important effector that controls protein synthesis, promoting cellular growth. This is one of the downstream targets of Akt, which phosphorylates and inactivates the product of tuberous sclerosis gene 2 (TSC2). Doing so, Akt counteracts the inhibition that TSC2 exerts over mTOR, thereby enhancing its growth promoting effect[19]. This effect is mediated by the phosphorylation of the ribosomal S6 kinase (S6K), that once activated phosphorylates and activates the ribosomal protein S6[32]. In addition, the translation initiation factor-4E binding protein-1 (4E-BP1) sees its activity increased by mTOR[6]. The net effect is an increased protein synthesis, with consequent cellular hypertrophy[6]. The facts that mice lacking Akt1 showed lower levels of phosphorylated S6K and S6 after exercise training[19] and that overexpression of IGF-1R was associated with an increase in phosphorylated S6K[25] highlight the importance of the IGF-1R/PI3K/Akt pathway for the induction of this system. However, the absence of S6K did not

attenuate cardiac physiological hypertrophy when mice were subjected to an exercise protocol, which suggests that, while important, S6K is not essential for the development of physiological hypertrophy[32].

Another mean by which Akt induces myocyte hypertrophy is by inactivation of glycogen synthase kinase-3 beta (GSK-3β)[19]. GSK-3β phosphorylates and inactivates the transcription factor GATA-4, thereby impairing protein synthesis[33]. Indeed, increased levels of the inactive phosphorylated form of GSK-3β were detected in mice overexpressing Akt, which reflected in the bigger accumulation of GATA-4 in cardiomyocytes' nuclei[30]. Once in the nucleus, GATA-4 regulates the expression of a variety of cardiac genes related with myocardium hypertrophy[33]. Moreover, GATA-4 has also been connected to myocyte proliferation in zebrafish[34]

Besides GATA-4, several other transcription factors have been associated to cardiomyocytes hypertrophy[33]. One of the identified transcription factors was C/EBPβ, whose expression was found to be reduced after exercise training. Interestingly, that fall was secondary to a higher Akt1 activity, and resulted in a reduction of the repressive effect that C/EBPβ has over the serum response factor (SRF). SRF is also a transcription factor that, among others, promotes the expression of GATA-4. Another downstream target negatively regulated by C/EBPβ is the CBP/p300-interacting transactivator with ED-rich carboxy-terminal domain 4 (CITED4). In fact, CITED4 was markedly increased in cardiomyocytes and induced its proliferation, as reflected by an increase in the number of cells[18].

The role of microRNAs

MicroRNAs (miRNA) are a class of recently discovered small molecules, each composed of a noncoding sequence of nearly 22 nucleotides, which can modulate gene

expression by destroying or repressing the translation of a target RNA. Each miRNA molecule can target more than a different mRNA, and each mRNA can be targeted by more than a different miRNA, which provides a fine control of gene expression[35]. Some miRNAs have already been identified that are known to play important roles in cardiac development. Namely, miRNA-1 and miRNA-133 are of uttermost importance in cardiac development by regulating the proliferation and differentiation of cardiomyocytes[36]. The role of miRNA-1 and miRNA-133 in adult heart is less certain, but both pathological and physiological hypertrophic stimuli decrease the expression of both miRNAs, which suggests an inverse correlation between miRNA-1 and miRNA-133 and myocardium hypertrophy[37].

In an attempt to identify miRNAs regulated by PI3K, Lin *et al*. found that mice expressing constitutively active PI3K (caPI3K) expressed lower levels of miRNA-222, miRNA-34a and miRNA-210 as opposed with controls. Moreover, although the levels of these miRNAs increased after myocardial infarction, they remained significantly lower in the group expressing caPI3K, and this was reflected in the improved cardiac function shown in this group[38]. Another miRNA that has been shown to be related with physiological hypertrophy following a swim training programme was miRNA-29c. However, unlike the former, miRNA-29c levels were upregulated after a programme of several weeks of physical exercise. The consequence was a downregulation in the expression of COLIAI and COLIIIAI, with a decrease of total left ventricle collagen content and improved ventricular compliance[39].

Endogenous Cardiac Stem Cells

As described above, the heart harbours a pool of endogenous cardiac stem cells (eCSC). The fact that exercised animals' hearts have shown evidence of new cardiomyocytes

formation raises a question – what is the relationship between physical exercise and eCSC?

Some studies exist that try to answer this question. In one of them, the number of c-Kit^{pos} eCSC was found to be increased in exercised rats in comparison with controls[22]. Also the IGF-1R/PI3K/Akt pathway has been connected with eCSC. In this field, current evidence points to a positive correlation between the activation of this pathway and the activation and proliferation of eCSC[22, 40]. Moreover, IGF-1 overexpression increased the activity of telomerase, which resulted in a delay in eCSC senescence[41]. Therefore, if the activation of IGF-1R/PI3K/Akt fosters the activation and proliferation of eCSC, and if physical exercise promotes the activation of the IGF-1R/PI3K/Akt signalling pathway, so it is reasonable to hypothesize that physical exercise is an effective trigger of eCSC activation and consequent cardiac regeneration.

Clinical insights

So far, the only available therapeutic options aim to block the progression on the heart disease[38]. However, as noted above, the last decade brought crescent scientific evidence that supports the possibility of exploring new therapeutic approaches that can improve the function of the failing heart, thereby improving the quality of life of millions of people suffering from cardiac pathology.

IGF-1R/PI3K/Akt pathway. As stated above, the IGF-1R/PI3K/Akt pathway is the central core of physiological hypertrophy. In fact, some studies have already been performed in the setting of myocardial infarction that showed a reduction in cardiac fibrosis, cardiomyocytes apoptosis and infarction size, as well as better systolic and diastolic function, following the administration of IGF-1[42, 43]. Furthermore, in animals subjected to myocardial infarction, the treatment with an association of IGF-1 and

hepatocyte growth factor (HGF) fostered eCSC activation with consequent cardiac regeneration[40, 44, 45]. Interestingly, this was observed along with increased expression of GATA-4[44] which, as described above, seems to have a role in physiological hypertrophy induced by physical exercise.

microRNAs. The discovery of miRNAs has brought a new biological target for future therapies. Actually, today is already possible either to increase the expression, using vectors[46], or antagonize specific miRNAs using 'antagomirs', which are cholesterol-associated RNAs that block the action of miRNAs over their targets[47]. The expression of miRNA-34a, aforementioned to be decreased in the cardiomyocytes of mice expressing caPI3K, has been shown to rise in the ageing heart and following myocardial infarction. Interestingly, using antigomir against miRNA-34a it was possible to observe a drop in cell death and cardiac fibrosis, and consequent improvement in contractile function in mice subjected to myocardial infarction[48]. Another miRNA described above as being negatively correlated with PI3K activity was miRNA-210. By those findings, it would be reasonable to think that a decrease in the expression of this miRNA would be beneficial. However, other studies found that miRNA-210, whose expression is upregulated under hypoxic conditions[49], is actually beneficial in the setting of myocardial infarction by significantly inducing angiogenesis and decreasing myocytes' apoptotic rate, thereby contributing for a more favourable ventricular remodelling and cardiac function[46].

Cell therapy. The first attempts to regenerate myocardial tissue have been done through transplant of stem cells from other origins than the heart[50, 51]. Although animal studies have provided exciting results, several clinical trials in humans where already performed and showed only modest, if any, improvement in cardiac function[50].

Therefore, new approaches are needed in order to make this type of therapy more effective.

One of the first questions to be answered is which type of cells suits better for cell-based therapy. The discovery of a pool of resident cardiac stem cells has opened a new window regarding the regenerative potential of this organ, which can be further explored either as a therapeutic tool or target. Indeed, a phase 1 clinical trial has documented significant improvements in both the ejection fraction and infarct size in patients that received autologous cardiac stem cells therapy compared with controls. Furthermore, the safety of the treatment was absolute, with no adverse effects registered in any of the treated patients[52].

The pool of cardiac stem cells is complex and comprises cells with different capacity to replicate and form mature myocytes and blood vessels. In an attempt to better characterize those different cellular populations, D'Amario *et al*. concluded that cardiac stem cells expressing IGF-1R were younger and had a better replicative reserve, as opposed with cells expressing IGF-2R and AT1R. Moreover, cells expressing IGF-1R were capable of secreting both IGF-1 and IGF-2, the latter being responsible for the induction of myocyte differentiation. The superiority of IGF-1R expressing cardiac stem cells was further confirmed by the greater degree of cardiac recovery after the transplant of these cells into hearts of rats subjected to myocardial infarction[53].

Besides the choice of the ideal cell, other strategies can be taken to maximize the beneficial effects of cell therapy. Interestingly, the combination of cardiac stem cells administration and nanofibers containing IGF-1 resulted in a higher myocardial recovery after ischemia when compared with each therapy alone[54].

Discussion

In the last decade several steps have been taken in order to open new lines of investigation regarding new therapeutic strategies. Maybe the biggest advances have been done in the field of cell therapy, with some clinical trials already in progress. However, as stated above, there is also growing expectation about miRNAs and its manipulation.

The central role that the IGF-1R/PI3K/Akt has in the development of physiological hypertrophy has also triggered attempts to modulate this signalling system, namely through the therapy with IGF-1. In fact, the results obtained with IGF-1 administration in animal models of myocardial infarction were encouraging[42, 43]. However, this signalling pathway is present in a wide range of cell types and because of that systemic administration of this growth factor can have diffuse effects and result in the progression of occult neoplasms[31].

Concerning cell therapy, some obstacles have been raised regarding the type of cells that better fulfil the requirements for the success of this therapeutic approach. By one side, the application of cells from other origins than the heart has shown disappointing results. Among the causes advanced for this is the source and inadequate preparation of the cells, as well as the timing and mode of cell delivery. On the other side, it is believed that embryonic stem cells are the ones that have the greatest potential to be successful. However, to the risk of teratoma formation and immunogenic incompatibilities that the use of embryonic stem cells entails, we still have to add the ethical issues[50]. Finally, it appears that autologous cardiac stem cells transplantation may be the best approach, as has been shown by a recent phase 1 clinical trial[52]. Still, more research is needed to find new strategies that can increase the feasibility and effectiveness of cell therapy.

The discovery of miRNAs has provided a new overview of cellular regulation mechanisms. In addition, it represents another process where it is possible to act to change the fate of diseased heart. Currently, it is already clear that different miRNAs are important in both the heart development and pathology[55]. Furthermore, some studies performed in animals were successful in improving the pattern of cardiac remodelling after myocardial infarction[46, 48]. Nevertheless, miRNAs represent a class of molecules that exerts a very delicate control of specific genes at a post-transcriptional level[55]. Consequently, it is critical to better understand the function of each specific miRNA in the heart's physiology and pathology, and only after it will be possible to delineate the best plans to take all the potential from this promising area.

Heart diseases, namely those of ischemic cause, are a major source of morbidity and mortality worldwide[51]. Despite the improvement that the development of several classes of drugs have brought to the prognostic of these patients, advances are need so that it can be possible to further decrease the burden of disease of cardiac pathologies.

Conflict of interest

None declared.

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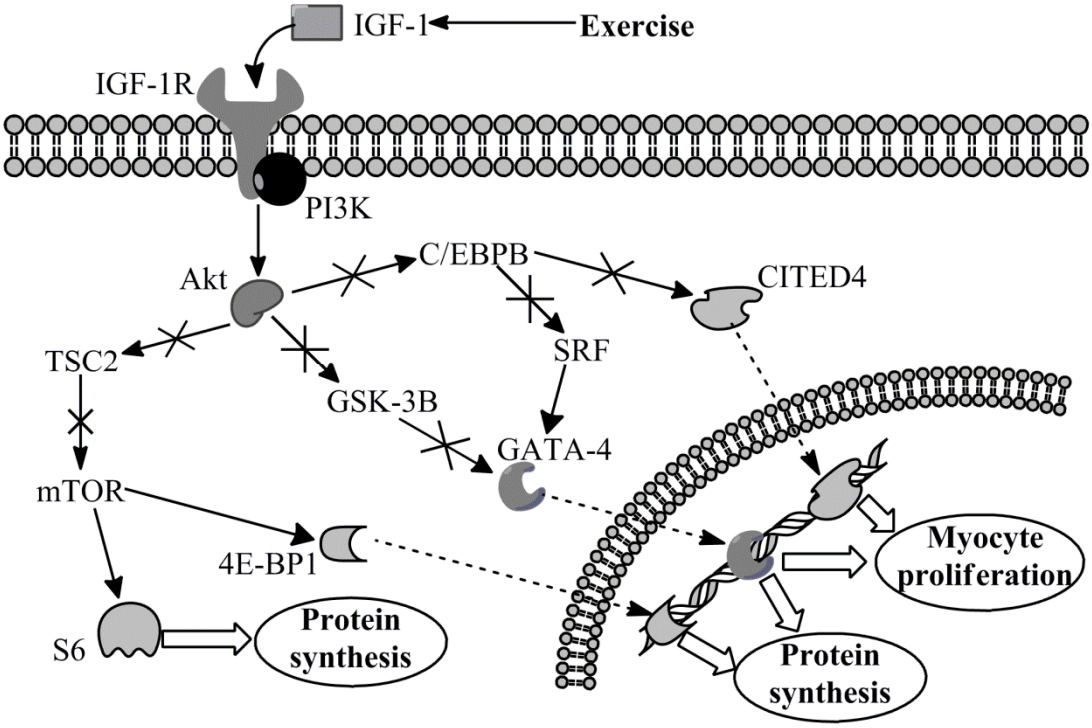
Figure legends

Figure 1 – IGF-1R/PI3K/Akt signalling pathway and downstream targets. Arrows mean activation; arrows with a cross mean inhibition; C/EBPB – C/EBPβ; GSK-3B – GSK-3β. For more details consult the text.

Figure 1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Exercise
IGF-1R
PI3K
Akt
C/EBPB
CITED4
TSC2
SRF
GSK-3B
GATA-4
mTOR
4E-BP1
S6
Protein synthesis
Myocyte proliferation
Protein synthesis

ANEXO

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