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MUSCLE WASTING AND CARDIAC MUSCLE DAMAGE IN CACHECTIC PATIENTS

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SUMMARY

Muscle wasting is a degeneration of the muscle tissue that can derive from several pathological situations, but most of the times is caused by a condition of cachexia in patients with cancer or other diseases. This degeneration results from a decrease in protein synthesis and an increase in protein degradation. This is caused mainly by the overexpression of ubiquitin-proteosome-system (UPS) elements, under the control of factors released in cachexia that lead cells toward a catabolic rather than an anabolic pathway. Both skeletal and cardiac muscles can be affected by muscle wasting and until now an effective treatment is unknown. Only experimental trials of exercise training bring to a recovery of mass loss, but many researchers think that a potential future treatment may be represented by stem cells.

Introduction

Muscle wasting is a weakening, shrinking, and loss of muscle caused by a disease or lack of use. It causes a decrease in strength and inability to move compromising the quality of life. Muscle wasting can be caused by cachexia, sarcopenia or anorexia. Cachexia is a condition associated with a number of chronic diseases and acute medical conditions where patients are often hospitalized or become extremely inactive. Some of the diseases that bring to cachexia are amyotrophic lateral sclerosis, multiple sclerosis, muscular dystrophy, neuropathy, polio, spinal muscular atrophy, cancer, acquired immunodeficiency syndrome (AIDS), congestive heart failure, chronic obstructive pulmonary disease (COPD), renal failure and other illnesses that lead to a chronic inflammation state (1). Sarcopenia lies in aging, it shows a decrease in skeletal muscle mass due to decreased physical activity and production of anabolic hormones. Anorexia results by lower energy intake often due to loss of appetite. Malnutrition can be present in cachexia. However, unlike other diseases related to this cause, cachexia represents a disturbed metabolic panel and it cannot be treated with nutrition alone (1).

Cachexia, sarcopenia and anorexia may cause both muscle wasting and damage to the cardiac muscle. In this review we point out our attention on muscle wasting and heart failure mainly caused by cachexia.

Definition of cachexia

The definition that scientists and clinicians agreed in the cachexia consensus conference in 2007 in Tampa (FL) describes cachexia as a "complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss off fat mass" (2).

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Cachexia may be caused by chronic infection, nutritional deficiencies, heart failure, rheumatoid arthritis, COPD, parasitic diseases, disease of the gastrointestinal system that lead to abnormal assimilation of food; but the mainly known cause of cachexia is cancer, which is responsible for the deaths of 22% of cancer patients. Cancer cachexia compromises quality of life and responses to anticancer therapies that result more toxic for patient, reducing survival.

Muliawati et al. (3) in a clinical study distinguished two kinds of cachexia: primary and secondary. The first is produced by a tumor-induced metabolic change; where catabolism is accelerated and anabolism slowed. Cancer triggers a systemic inflammatory response that releases biochemical products. Everything brought to metabolic abnormalities, loss of appetite, and decrease of muscle and fat mass. The secondary cachexia is caused by factors that compromise the normal food intake leading to malnutrition, nausea, vomiting, diarrhea and mechanical obstruction. In cancer cachexia, in general, there is a scenery similar to tissue inflammation: the liver protein synthesis switches from synthesis of albumin to acute-phase proteins (c-reactive protein, fibrinogen α1-antitrypsin). In this kind of cachexia, the acute-phase response is activated by cytokines such interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) produced by host tissues or tumor cells; it does not involve loss of weight, and it is not sufficient to cause cachexia (4).

Skeletal muscle wasting in cachexia

This derangement involves muscle proteins and is characterized by a decrease in protein synthesis and an increase in protein catabolism.

Protein synthesis is regulated by two key factors: eukaryotic initiation factor 2 (eIF2) and factor 4F (eIF4F). The first is associated with a pathway GTP/GDP-dependent and leads to an active translation, while there is a block of translation when eIF2 is phosphorylated. The latter drives to a correct starting of the translation; it results from the cooperation among eIF4E-4A-4G. In a cachectic subject there is an increase in the phosphorylation of eIF2 (5) and binding of eIF4E to 4E-BP1 (6), the constitutive regulator of eIF4F complex.

Protein catabolism may be due to the activation of the lysosomal system, cytosolic calcium-regulated calpains and mainly to the ATP ubiquitin-dependent proteolytic pathway. This degradation brings to loss of muscle fibers and later to atrophy. The lysosomal system is mainly responsible for the degradation of extracellular proteins and receptors. Pathways involving calpains attend in tissue injury and necrosis. The ubiquitin-proteasome pathway contributes to the degradation of myofibrillar proteins (7).

The release of superoxide anions, hydrogen peroxides or nitric oxide can contribute to muscle wasting. These molecules can interact each other and generate more highly reactive products (Peroxynitrite and hydroxyl radical). This series of events can set off by an inflammatory event and they culminate in oxidative stress (8). The increase of reactive oxidative species (ROS) promotes catabolism of muscle cells even more: study conducted in C2C12 cells documented that ROS induce an up-regulation of E3-ubiquitin ligases (9), and accordingly grow up of proteasome activity and degradation of myosin. In cancer cachexia myosin heavy chain (MyHC) is an important target of muscle wasting. It is reduced in protein form but steady in mRNA level, as it was established with an experiment of Lenk et al. (10) where MyHC immunoprecipitated with ubiquitin.

The fast-twitch type II fibers (in tibialis anterior and gastroecnemius) are lost faster than slow-twitch type fibers (in soleus). The reason is an increased degradative protein expression and protein oxidation with decrease in anti-oxidant gene expression (11). In skeletal muscle there are two muscle-specific E3 ligases: atrogin-1 and MuRF1(12). Both act on myofibrillar and intracellular proteins, labeling them for degradation through the ubiquitin-proteosome system. Expression of atrogin-1 and MuRF1 are regulated by IGF-I/PI3K/Akt pathway, FoxO1 and 3, NFkB (12).

Indeed muscle wasting is a direct consequence of circulating proteins produced by both the host and tumour.

Among these, a considerable factor is forkhead box class O (FoxO). In myotube, activation of FoxO3 stimulates protein degradation both lysosomal and proteasome-dependent (11). Glucocorticoids contribute to FoxO activation decreasing

action of PI3K/Akt pathway that inactivates it by phosphorilation (10). It was highlighted in a study by Liu et al (13) on a cancer cachexia mice model, where the expression of FoxO1 in normal and cachectic mice was reduced using silencing oligonucleotides: this caused an increase in skeletal muscle mass of the mice, an increase in the levels of MyoD and a decrease in the levels of Myostatin.
Myostatin, a TGFβ suparfamily member, is the responsible factor for stopping growth of muscle tissue. Murine and human neoplasms further secrete it. It seems that myostatin is involved in the generation of cancer cachexia. Murine C26 colon cancer cells release a vast amount of myostatin, and experiments conducted with C2C12 exposed to C26 conditional medium demonstrated that the tumour mass produces such an amount of myostatin that can trigger skeletal muscle wasting (14). Probably because it blocks proliferation and differentiation of the cells, and induces a decrease in MyoD levels. Skeletal muscle in C26 tumour bearing mice also displayed elevated activity of the autophagy-lysosome pathway. In light of this Lokireddy et al. (12) asserted that "myostatin is a novel tumoral factor" .
The loss of muscle mass is also affiliated to an increased serum level of a tumour produced proteolysis-inducing factor (PIF). Indeed an administration of PIF in normal mice brings to a rapid decrease in body weight and enhancement in mRNA of ubiquitin in the gastroecnemius and components 19S and 20S proteosome (15). PIF activates a pathway that involves phospholipase A_2 (PLA_2) that produces arachidonic acid (AA) that in turns actives NADPH oxidase. This mechanism releases ROS and activates nuclear factor kappa B (NFkB) contributing to proteasomic degradation downstream (10).
Also cytokines are involved in muscle wasting. Costelli et al. (16), using inhibitors for TNF-α and IL-6 in rats bearing hepatoma, they prevented the depletion of muscle mass and reduced proteolysis (Figure1).
Opposite to these negative factors, insulin-like growth factor-1 (IGF1) can positively regulate muscle mass stimulating protein synthesis.
A cachectic individual is also subjected to alterations in resting energy expenditure (REE) contributing to the energy deficit, therefore, to the wasting and the weight loss of the patients. Tumor presents low level of oxygen so the Krebs cycle and mitochondrial oxidative phosphorylation cannot have place. For this reason the great amount of glucose that tumor consumes is converted in lactate. The latter is after converted in glucose with Cory cycle (4).

Cardiac muscle damage in cachexia

Also the cardiac muscle may be damaged by cachexia. A statement of this assertion is in the study of Xu H et al. (17). In this research 24 CD2F1 mice were inoculated with an injection of colon 26 adenocarcinoma cell suspension. Tumor was evident after seven days, and mice begin to die by

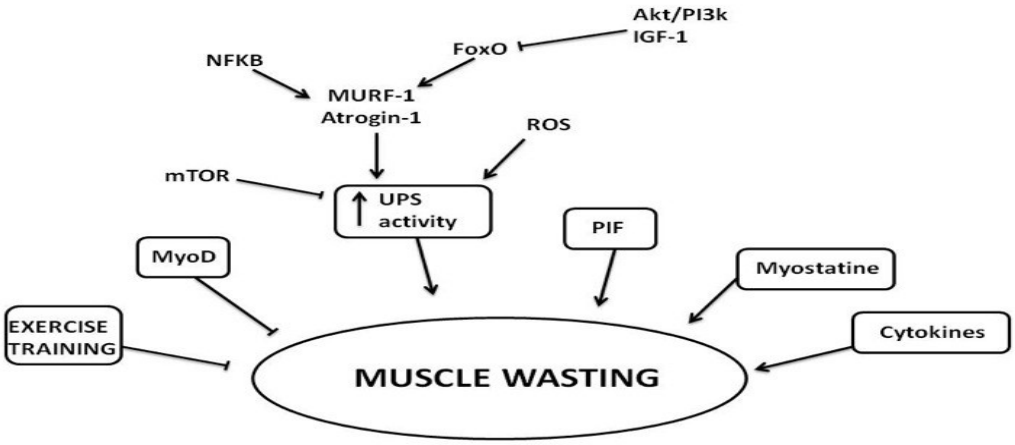

Figure 1. Skeletal muscle wasting in cachexia

day 21. This experiment resulted in a decrease in the weight of gastroecnemius while heart weight remained stable; on the contrary, the expression of MAFbx and Bnip3 mRNAs was higher in tumor-bearing mice compared to controls. Similarly, left ventricular systolic diameter (LVSD) was increased, while diastolic posterior wall thickness was decreased significantly in tumor-bearing mice. Moreover, cardiomyocytes isolated from tumor-bearing mice showed increased sarcomere contraction.

Treatments

The first attempt to reduce cachexia effects on lean mass reduction was to use appetite stimulants such megestrol acetate, however gain in weight was due to an accumulation of fat rather than body mass (11). Another possibility was cyproheptadine, a histamine antagonist; it improved in appetite but did not avoid weight loss. Corticosteroids were also used to enhance appetite, performance and sensation of well-being but they did not have positive effects on body weight (18). Neuropeptides regulate appetite, but they are still examined to understand its importance in the treatment of cancer cachexia. We know that growth hormone (GH) influences in a positive manner muscle mass but its clinical efficacy has not been demonstrated yet (19).

Moreover cachexia can be treated with an inhibitor of gene transcription of TNF (Pentoxifylline), with anti-cytokine antibodies and cytokine receptor antagonists. Anti-inflammatory/anabolic cytokine, like interleukin-15 (IL-15), has a key role in a decrease in protein degradation and DNA fragmentation (20) .

Until now the only treatment that brings good results in recovery of loss mass is exercise training. It may reduce pro-inflammatory cytokine expression, mainly of TNF and IL-6 (19) and the expression of myostatin in skeletal muscle (21). Peak of VO_2 (maximal aerobic capacity) and muscle strength are also improved by exercise training (22). The anabolic component of exercise training involves the release of cytokine like IL-6, which inhibits TNF-α production, and it increases activation of antioxidant enzymes. Resistance training, moreover, induces significant phosphorylation on mTOR favouring protein synthesis (21).

A regular physical activity can improve quality of life and it has positive effects on the chance of survival. Probably the efficacy of exercise training is linked to the adult stem cells (ASC) of skeletal and cardiac muscle tissue.

Skeletal and cardiac muscle stem cells

In the skeletal muscle tissue there are undifferentiated cells between the basal lamina and the plasma membrane. These cells are called “satellite cells” (SC) able to proliferate and differentiate when it needs: indeed they are involved in muscle growth and they have the possibility to repair skeletal muscle fibers after injury (9).

Satellite cell frequency is different in the several fiber type muscle components. For example soleus muscle, predominantly composed of slow fibers, has more satellite cells than fast fibers of extensor digitorum longus (EDL) muscle. In the differentiation process of SC the MyoD family of the transcription factors comprehends mainly Myf5, MRF4, MyoD. The expression of the latter is absent in the quiescent phase, but it increases after external stimuli that actives cellular proliferation and differentiation. This signal is stopped by the interaction between MAFbx and MyoD, which led to proteasome degradation. For this reason MyoD-dependent differentiation is negatively influenced by atrophy and increased oxidative stress (9). An important proliferative and differentiation factor is Pax7 (23) that is down-regulated by myostatin. Notch and bFGF also stimulate proliferation (23) while myogenin and MRF4 promote differentiation.

Between cachexia and skeletal muscle SC there seems to be a strict correlation. Very recently it has been suggested that cachexia induced by congestive heart failure (CHF) and chronic kidney disease (CKD) may be caused by an increase in the levels of angiotensin II (Ang II) which reduces skeletal muscle regeneration via inhibition of satellite cell (SC) proliferation (24).

On the other hand an exercise intervention increases the number of ASC in the muscle 5-8 days after training (25,26). Muscle regeneration is activated by a maximal eccentric contraction that recreates an environment similar to an injury. Macaluso et al. (27) performed a study with healthy young male individuals, in the attempt to demonstrate that there is a different

amount of SC in the muscles of sedentary and active individuals. From this observation resulted that the higher stress induced by a daily training in individuals with low VO_2 max the amount of differentiated stem cells was increased, while the SC pool size was decreased. These conditions were probably associated with a higher basal activation of p38-mitogen-activated protein kinase. This result probably derived from the self-renewal capacity activated by both exercise training stress and injury. Hence, considering that in a cachectic patient SC are impaired, exercise training may reduce muscle wasting counteracting the reduction in the proliferation state of SC.

Despite many studies deal about skeletal stem cells, there are a few witnesses about cardiac stem cells yet. Until 90' years, the scientific world thought that cardiomyocytes were terminal cells without possibility to regenerate. Despite this Beltrami et al. in 2003 (28) isolated a little percentage of cardiac cells that showed a cardiac phenotype both in vitro and in vivo. These cardiac ASC have been widely studied by many research groups and ourselves (29-35), but their biology, immunological features, differentiation pathways are still unknown. They are c-Kit-Sca1-MDR1 positive, they express cardiac troponin T and several cardiac sarcomeric proteins, typical of cardiac precursor cells (34). But also they express CD49, Connexin 43, Troponin T, OCT-4 and Nanog that are typically cardiac markers (36). Other studies demonstrated that these cells express also CD31 and CD34 like endothelial progenitor cells (EPC), but only in the first three weeks from isolation (37).

While a daily training may induce an increase in the number of SC in the skeletal muscle, it is not known if it can induce also an increase in the number of cardiac progenitor cells in the myocardium, even if it may be supposed (26). We can expect that a physical activity could give benefits also in cardiac tissue, seen the common features between skeletal and cardiac tissue and the stimuli received by the cardiac tissue during exercise training.

Conclusions

Muscle wasting of skeletal and cardiac tissues is an effect of cachexia, no cure exist for this at the moment. Only exercise training may ameliorate damages induced by cachexia like recovery of muscle mass. For this reason it would be desirable to understand the mechanisms underlying this phenomenon in order to recreate a possible pharmacological treatment that can mime its effects (exercise mimicking). Considering literature, one of the mechanisms underlying recovery of muscle mass upon exercise in cachectic patients could be proliferation of ASC (38). This let us suppose that ASC may be used directly to replace damaged muscle fibers in cachectic patients, as many researcher's groups are trying to do in clinical trials for muscular dystrophies or cardiac diseases.

References

1.Evans WJ, Morley JE, Argilés J, Bales C, Baracos V, Guttridge D, Jatoi A, Kalantar-Zadeh K, Lochs H, Mantovani G, Marks D, Mitch WE, Muscaritoli M, Najand A, Ponikowski P, Rossi Fanelli F, Schambelan M, Schols A, Schuster M, Thomas D, Wolfe R, Anker SD: Cachexia: a new definition. Clin Nutr 2008; 27:793-9.

2.Coletti D, Adamo S: complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss off fat mass. Basic Appl Myol 2008; 18:109-114.

3.Muliawati Y, Haroen H, Rotty LW: Cancer anorexia - cachexia syndrome. Acta Med Indones 2012; 44:154-62.

4.Tisdale MJ: Molecular pathways leading to cancer cachexia. Physiology (Bethesda) 2005; 20:340-8.

5.Eley HL, Russell ST, Tisdale MJ: Effect of branched-chain amino acids on muscle atrophy in cancer cachexia. Biochem J 2007; 407:113-20.

6.Eley HL, Tisdale MJ: Skeletal muscle atrophy, a link between depression of protein synthesis and increase in degradation. J Biol Chem 2007; 282:7087-97.

7.Hasselgren PO, Wray C, Mammen J: Molecular regulation of muscle cachexia: it may be more than the proteasome. Biochem Biophys Res Commun 2002; 290:1-10.

8.La Rocca G, Di Stefano A, Eleuteri E, Anzalone R, Magno F, Corrao S, Loria T, Martorana A, Di Gangi C, Colombo M, Sansone F, Patanè F, Farina F, Rinaldi M, Cappello F, Giannuzzi P, Zummo G. Oxidative stress induces myeloperoxidase expression in endocardial endothelial cells from patients

with chronic heart failure. Basic Res Cardiol. 2009;104:307-20.
9.Li YP, Chen Y, Li AS, Reid MB: Hydrogen peroxide stimulates ubiquitin-conjugating activity and expression of genes for specific E2 and E3 proteins in skeletal muscle myotubes. Am J Physiol Cell Physiol 2003; 285:C806 12.
10.Lenk K, Schuler G, Adams V: Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. J Cachexia Sarcopenia Muscle 2010; 1:9-21.
11.Tisdale MJ: Mechanisms of cancer cachexia. Physiol Rev 2009; 89:381-410.
12.Lokireddy S, Wijesoma IW, Bonala S, Wei M, Sze SK, McFarlane C, Kambadur R, Sharma M: Myostatin is a novel tumoral factor that induces cancer cachexia. Biochem J 2012; 446:23-36.
13.Liu CM, Yang Z, Liu CW, Wang R, Tien P, Dale R, Sun LQ: Effect of RNA oligonucleotide targeting Foxo-1 on muscle growth in normal and cancer cachexia mice. Cancer Gene Ther 2007; 14:945-52.
14.Zhou X, Wang JL, Lu J, Song Y, Kwak KS, Jiao Q, Rosenfeld R, Chen Q, Boone T, Simonet WS, Lacey DL, Goldberg AL, Han HQ: Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. Cell 2010; 142:531-43.
15.Lorite MJ, Smith HJ, Arnold JA, Morris A, Thompson MG, Tisdale MJ: Activation of ATP-ubiquitin-dependent proteolysis in skeletal muscle in vivo and murine myoblasts in vitro by a proteolysis-inducing factor (PIF). Br J Cancer 2001; 85:297-302.
16.Costelli P, Bossola M, Muscaritoli M, Grieco G, Bonelli G, Bellantone R, Doglietto GB, Baccino FM, Rossi Fanelli F: Anticytokine treatment prevents the increase in the activity of ATP-ubiquitin- and Ca(2+)-dependent proteolytic systems in the muscle of tumour-bearing rats. Cytokine 2002; 19:1-5.
17.Xu H, Crawford D, Hutchinson KR, Youtz DJ, Lucchesi PA, Velten M, McCarthy DO, Wold LE: Myocardial dysfunction in an animal model of cancer cachexia. Life Sci 2011; 88:406-10.
18.Tisdale MJ: Clinical anticachexia treatments. Nutr Clin Pract 2006; 21:168-74.
19.Osterziel KJ, Strohm O, Schuler J, Friedrich M, Hänlein D, Willenbrock R, Anker SD, Poole-Wilson PA, Ranke MB, Dietz R: Randomised, double-blind, placebo-controlled trial of human recombinant growth hormone in patients with chronic heart failure due to dilated cardiomyopathy. Lancet 1998; 351:1233-7.
20.Smart NA, Steele M: The effect of physical training on systemic proinflammatory cytokine expression in heart failure patients: a systematic review. Congest Heart Fail 2011; 17:110-4.
21.Lenk K, Erbs S, Höllriegel R, Beck E, Linke A, Gielen S, Winkler SM, Sandri M, Hambrecht R, Schuler G, Adams V: Exercise training leads to a reduction of elevated myostatin levels in patients with chronic heart failure. Eur J Prev Cardiol 2012; 19:404-11.
22.Hsieh PL, Wu YT, Chao WJ: Effects of exercise training in heart transplant recipients: a meta-analysis. Cardiology 2011; 120:27-35.
23.Chen JC, Goldhamer DJ: Skeletal muscle stem cells. Reprod Biol Endocrinol 2003; 1:101.
24.Yoshida T, Galvez S, Tiwari S, Rezk BM, Semprun-Prieto L, Higashi Y, Sukhanov S, Yablonka-Reuveni Z, Delafontaine P: Angiotensin II Inhibits Satellite Cell Proliferation and Prevents Skeletal Muscle Regeneration. J Biol Chem 2013; 288:23823-32.
25.Macaluso F, Brooks NE, Niesler CU, Myburgh KH: Satellite cell pool expansion is affected by skeletal muscle characteristics. Muscle Nerve 2013; 48:109-16.
26.Crameri RM, Langberg H, Magnusson P, Jensen CH, Schrøder HD, Olesen JL, Suetta C, Teisner B, Kjaer M: Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise. J Physiol 2004; 558:333-40.
27.Macaluso F, Brooks NE, van de Vyver M, Van Tubbergh K, Niesler CU, Myburgh KH: Satellite cell count, VO(2max) , and p38 MAPK in inactive to moderately active young men. Scand J Med Sci Sports 2012; 22:e38-44.
28.Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P: Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003; 114:763-76.
29.Di Felice V, Zummo G: Tetralogy of fallot as a model to study cardiac progenitor cell migration and differentiation during heart development. Trends Cardiovasc Med 2009; 19:130-5.
30.Di Felice V, De Luca A, Colorito ML, Montalbano A, Ardizzone NM, Macaluso F,

Gammazza AM, Cappello F, Zummo G: Cardiac stem cell research: an elephant in the room? Anat Rec (Hoboken) 2009; 292:449-54.
31.Di Felice V, Ardizzone NM, De Luca A, Marcianò V, Marino Gammazza A, Macaluso F, Manente L, Cappello F, De Luca A, Zummo G: OPLA scaffold, collagen I, and horse serum induce an higher degree of myogenic differentiation of adult rat cardiac stem cells. J Cell Physiol 2009; 221:729-39.
32.Di Felice V, De Luca A, Serradifalco C, Di Marco P, Verin L, Motta A, Guercio A, Zummo G: Adult stem cells, scaffolds for in vivo and in vitro myocardia tissue engineering. Ital J Anat Embryol 2010; 115:65-9.
33.Serradifalco C, Catanese P, Rizzuto L, Cappello F, Puleio R, Barresi V, Nunnari CM, Zummo G, Di Felice V: Embryonic and foetal Islet-1 positive cells in human hearts are also positive to c-Kit. Eur J Histochem 2011; 55:e41.
34.Di Felice V, Zummo G: Stem cell populations in the heart and the role of Isl1 positive cells. Eur J Histochem 2013; 57:e14.
35.Di Felice V, Serradifalco C, Rizzuto L, De Luca A, Rappa F, Barone R, Di Marco P, Cassata G, Puleio R, Verin L, Motta A, Migliaresi C, Guercio A, Zummo G. Silk fibroin scaffolds enhance cell commitment of adult rat cardiac progenitor cells. J Tissue Eng Regen Med 2013; doi: 10.1002/term.1739.
36.Minervini F: Le cellule staminali adulte cardiache: mito o realtà? Cardiac adult stem cells: myth or reality. Capsula Eburnea 2006; 1: 1250.
37.Wagner SJ, Myrup AC: Toward closed-system culture of blood origin endothelial cells. Transfusion 2005; 45:1201-7.
38.Macaluso F, Myburgh KH: Current evidence that exercise can increase the number of adult stem cells. J Muscle Res Cell Motil 2012; 33:187-98.