Chronic heart failure and proinflammatory cytokines: possible role of physical exercise

Running Title: Exercise and CHF-related inflammation

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Introduction

Chronic heart failure (CHF) is characterized by the inability of the heart to supply the body with sufficient amount of blood for metabolic and circulatory needs. Characteristically, this progressive evolving syndrome is accompanied by local and systemic adaptive processes which first compensate for the decreased cardiac function and preserve systemic perfusion. Beyond neurohumoral activation, functional alterations include endothelial dysfunction and structural changes such as peripheral myopathy which in turn impair the functional capacity of patients with heart failure, contributing to progressive aggravation of CHF symptoms in the long term. In this respect it became obvious that continued activation of the neuro-endocrine system while being initially adaptive, exerts detrimental long-term effects both in terms of symptoms in patients with CHF but probably also in terms of the prognosis of these patients. Thus, it is our current view that persistent activation of these systems are maladaptive. Accordingly, inhibition of the renin-angiotensin-aldosterone-system by angiotensin-converting enzyme (ACE)-inhibitors and inhibition of sympathetic tone by ß-blockers have been shown to improve the clinical status of CHF patients, their prognosis and do represent now a hallmark of therapy in patients with heart failure. Yet, important aspects of their mechanism of action in heart failure are still unclear. It has been demonstrated however, that long-term treatment with ACE inhibitors leads to improvement of peripheral myopathy, endothelial function and peripheral oxygen utilization.

Apart from this neuroendocrine activation heart failure is characterized by an inflammatory component. There is accumulating evidence that inflammation plays an important role in the development of left ventricular remodelling including structural and functional changes of the myocardium. Notably, mediators of inflammation act also as catabolic factors that are thought to be involved in the pathogenesis of peripheral muscle wasting and cardiac cachexia. Peripheral myopathy in CHF appears to develop independently of the haemodynamic changes caused by reduced left ventricular function. It turned out that sustained activation of pro-inflammatory factors are deleterious and maladaptive in the

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long-term (similar to persistent neuroendocrine activation). Nevertheless, these inflammatory processes seem to be adaptive in acute phases of ischaemia, thereby providing protective effects.

There is now some evidence that both ACE-inhibitors and physical training not only block neuroendocrine systems but, also depress proinflammatory processes in the circulation and possibly in skeletal muscle itself. For example, it was shown that systematic physical exercise was able to break the vicious circle of neuroendocrine- and inflammatory- stimulation of the skeletal and respiratory muscle in CHF and consequently improve exercise intolerance as one of the key symptoms by reducing peripheral markers of inflammation. It is quite obvious that inflammation is a key feature of chronic heart failure. While targeting specific pro-inflammatory factors such as TNF has not been beneficial so far in patients with heart failure in large trials, physical exercise is associated with significant depression of pro-inflammatory cytokines raising the possibility that reduction of cytokine activation may represent an important mechanism by which exercise exerts its beneficial effects.

Inflammation in the failing myocardium

Inflammation requires leukocytes to be present in the tissue, in this case in the myocardium. In this respect, it has been shown that the myocardium in patients with dilative cardiomyopathy shows a phenotypical pattern of inflammation with infiltration of mononuclear cells (1). In addition, increased mast cell density has been reported in the myocardium of both patients with dilative cardiomyopathy and ischaemic cardiomyopathy. Thus the increase in mast cells in cardiac tissue is not a consequence of myocarditis but found in CHF per se. Release of inflammatory mediators from cardiac mast cells caused by immunological and nonimmunological stimuli appears to be higher in patients with dilative and ischaemic cardiomyopathy compared with control subjects (2). Do these increased numbers of mast cells play a functional role ? There is experimental evidence that mast cell deficient mice exposed to chronic pressure overload by aortic banding do not show signs of decompensated hypertrophy and signs of heart failure compared to their wildtype controls (3). Thus, mast cell mediated inflammation may be required for the transition to heart failure once these animals are exposed to pressure overload suggesting that inflammation is involved in the progression to heart failure.

Myocardial and systemic levels of cytokines

Cytokines are biologically active molecules which stimulate via specific receptors different processes such as gene expression and play important roles in signalling for immune modulation. A large number of different cytokines are known, which are generally characterised as pro-inflammatory or anti-inflammatory cytokines. Accumulating evidence strongly indicates that cytokines play a pathophysiological role as mediators of the immune system in different cardiac diseases, not only in myocarditis as an obviously inflammatory disease but also in CHF. Numerous observations suggest that these immune modulators are not only a consequence of reduced cardial contractility but play a causal role in the pathogenesis of CHF. In fact cytokines, in particular tumour necrosis factor alpha

(TNF-α), have been shown to exert cardiovascular effects which are typically found in heart failure, Thus, it is quite likely that cytokines are involved in the development of key features of clinical heart failure; i.e. TNFα exerts negative inotropic effects and left ventricular dysfunction, endothelial dysfunction, cardiomyopathy, pulmonary oedema, reduced skeletal muscle blood flow, anorexia and cachexia, cardiac myocyte apoptosis, and activation of the foetal gene program. These inflammatory mediators are now known to be expressed by all cell types residing in the myocardium, including the myocyte. While the increased expression and activity of pro-inflammatory cytokines is well documented, it is less clear what are the triggers for their activation in the heart. However, experimental studies have shown that myocardial ischaemia, angiotensin (i.e. an activated renin-angiotensin system) and pressure overload can induce cytokine expression in the myocardium.

Notably, cytokine concentrations are not only elevated in the myocardium, but also in the systemic circulation. Cytokines are not only thought to be involved in autocrine and paracrine effects of the myocardium but appear to act also as an endocrine effector in the peripheral circulation. The sources of the increased circulating levels of cytokines are not fully clarified and several possibilities have been proposed. While the heart may represent one source of cytokine production, it appears that the increased plasma levels are related also to other peripheral production sites. One concept is, that endotoxin-induced immune activation emerges secondary to the gastrointestinal oedema, followed by enhanced exposure to endotoxins with subsequent pro-inflammatory responses (4). Similarly, it has been hypothesised that prolonged physical activity and high level exercise training can be associated with increase exposure of endotoxins entering via the gut eliciting pro-inflammatory responses. In chronic heart failure, some studies suggest that impaired tissue perfusion and hypoxia may be involved in peripheral synthesis of cytokines, such as interleukin 6 (IL-6).

The levels of plasma cytokines, in particular IL-6 and TNF-α are related to the severity of heart failure as classified by NYHA classes (5). Moreover, there is a relationship between the plasma levels of TNF-α and survival in patient with CHF, i.e. the circulating levels of TNF-α are a strong independent predictor of prognosis, beyond haemodynamic and clinical parameters (6). Cytokines appear to be activated at early stages of CHF, arguing against the assumption that cytokine activation in heart failure is simply attributed to enhanced neurohumoral factors such as angiotensin (7).

Experimental studies have demonstrated that sustained cardiac expression of TNF-α mimics the key features of severe heart failure phenotype. For example infusion of pathophysiologically relevant doses of TNF-α lead to partially reversible left ventricular dysfunction (8). Transgenic TNF-α overexpressing mice develop left ventricular dysfunction and dilated cardiomyopathy. The degree and progress of dysfunction depends on the degree of overexpression (9). However, in contrast to the deleterious effect of increased TNF-α in the long term, short-term endogenous TNF-α can protect cardiomyocytes acutely against ischaemia induced apoptosis in the mouse model of myocardial infarction (10). Thus, short-term activation of endogenous TNF-α may provide protective effects in repetitive ischaemia in patients with ischaemic heart disease (the majority of CHF patients).

Notably, besides TNF-α, IL-6 is a strong independent predictor for survival

in patients with CHF (11). Moreover, data from the Framingham survey suggest that IL-6 can estimate the risk to develop heart failure in older individuals without previous history of cardiac events (12). Thus, IL-6 may not only represent a prognostic marker but also a risk factor for developing the disease CHF. These observations do not establish whether or not interleukin-6 is involved in the disease process or merely a marker of the disease. In other words, are high levels of IL-6 harmful in a pathophysiological sense or do they represent only a marker for the prognosis and progression of CHF? To address this issue we recently completed experimental studies using mice with systemic knockout of IL-6. In these series of studies, mice deficient for IL-6 had similar long-term survival, left ventricular function and remodelling after experimental myocardial infarction as compared to corresponding wild type mice (13). In mice lacking IL-6, other members of the IL-6 family such as LIF and other factors signaling via JAK/STAT such as angiotensin appeared to act in a compensatory manner to activate the JAK/STAT pathway, which is crucial for the cellular effects of IL-6 cytokines. The IL-6 family of cytokines leads to activation of several target genes by signalling via a membrane bound receptor (gp130) and activation of further down stream intracellular signalling proteins (JAK and STAT). In this respect we have recently shown that patients with dilative cardiomyopathy show increased levels of circulating IL-6 and increased activation of its specific receptor gp130. However, myocardial expression and activation of the consecutive JAK/STAT signal transduction pathway is impaired (i.e. JAK2 and STAT3) (14). In fact mice deficient for one of these signal molecules (STAT3) develop cardiomyopahty and heart failure with a marked increase in mortality (15). Thus, the signal transduction of IL-6 cytokines in the myocardium seems to be protective, while inhibition of the JAK-/STAT pathway is deleterious! Taken together, cytokine activation in heart failure may not necessarily be harmful in general which needs to be considered in the development of anti-inflammatory therapeutic interventions.

Cytokines and peripheral myopathy in heart failure

The alterations in skeletal muscle in patients with CHF include muscle atrophy, programmed cell death (apoptosis), increased fibre type switching from oxidative type I fibres to glycolytic type IIb fibres, decreases in mitochondrial enzymes involved in oxidation of fatty acids, decrease in cytochrome c oxidase and mitochondrial volume density (16,17). Many of these abnormalities cannot be explained solely by endothelial dysfunction and reduction in muscle blood flow or by prolonged inactivity (18).

TNF-α is related to cardiac cachexia in patients with severe CHF. Administration of TNF-α to animals has been shown to cause a reduction in the number of mitochondria and protein reserves in skeletal muscle, leading to cachexia (19). Cytokines cause muscle wasting through both indirect (anorexia) and direct (e.g. inhibition of protein synthesis, induction of iNOS, apoptosis) mechanisms. Thus TNF-α intensifies skeletal muscle catabolism by opposing the nutritive action of anabolic hormones like insulin on skeletal muscle cells (20), or by reducing the expression of growth factors such as insulin-like growth factor-1 (IGF-1) (21). TNF-α stimulates expression of inducible nitric oxide synthase (iNOS) via nuclear-factor-kappa-B (22). In fact we have recently shown that iNOS expression

is elevated in muscle biopsies of patients with CHF (23). Subsequently, excessive intracellular levels of nitric oxide may inhibit key enzymes of aerobic metabolism and therefore reduce peak oxygen uptake (24). In animal studies it has been show, that iNOS inhibitors prevented TNF-α induced muscle wasting (25). Moreover, TNF-α is thought to be involved in muscle wasting due to excessive activation of apoptosis. Programmed cell death in peripheral muscles is seen in nearly half of all patients with CHF, but not in healthy volunteers, leading to reduced exercise capacity in patients with apoptosis positive biopsies (26). Consequently peak oxygen consumption was inversely correlated with the number of apoptotic nuclei in skeletal muscle (27). TNF-α can provoke apoptosis in skeletal myotubes (21). The levels of circulating TNF-α correlated with the number of apoptotic nuclei in peripheral muscle in an experimental animal model of CHF (28,29).

Notably, muscle weakness can occur without loss of muscle protein in inflammatory diseases. In this respect it was shown that TNF-α can decrease contractile function in striated muscle (30,31). TNF-α overexpressing mice exert a profound weakening of diaphragm muscle force generation that was accompanied by evidence of increased cytosolic oxidative stress. In this mouse model of TNF-α overexpression, antioxidants prevented much of the force deficit suggesting that oxidative stress was responsible for contractile dysfunction (32). Inflammatory mediators such as TNF-α can increase the production of reactive nitrogen species by skeletal muscle fibres elevating intracellular oxidant levels and decreasing force of contraction (33).

Inflammation as a therapeutic target in CHF

If inflammation is involved in the pathophysiology of heart failure and progression of the disease, inhibition of increased levels of circulating cytokines and enhanced tissue expression should be beneficial in heart failure. In fact, some experimental studies suggested that antibodies against TNF may attenuate or even reverse key aspects of the failing heart. These observations suggested that the deletrious effects of inflammation can be prevented by inhibition of cytokine synthesis (inhibition of transcription and translation), antagonizing circulating cytokines.

Both pentoxyphylline and thalidomide have been used in patients with moderate to advanced CHF with some success in small studies. Both agents affect the systhesis of inflammatory mediators by blocking their transcriptional activation. Treated patients improved in symptoms and left ventricular function and importantly the level of circulating TNF-α decreased significantly (34, 35). The high plasma levels of TNF-α in CHF patients and the deleterious long term effects on the myocardium were the basis to use TNF-α antagonists. However, in contrast to the promising results in small clinical studies, (36), the large multicentre trials (RENAISSANCE in North America; RECOVER in Europe and Australia) using TNF-α antagonism by etanercept, a TNF antibody, did not affect morbidity and mortality in patients with NYHA class II-IV heart failure (37). Similarly, the ATTACH study (38), which used a chimeric monoclocal IgG1-antibody (mouse/human) (Infliximab) able to bind membrane bound as well as soluble TNF-α, was ineffective and resulted in a dose related excess rate of death. It should be noted that both TNF antibodies used in these studies do have significant limitations in their effectiveness to block the biological effect of TNF during

long-term therapy. Thus, the failure of anti TNF-α therapy in CHF patients should not be interpreted as an absence of an important role for TNF-α in the development and progression of CHF. Rather the study design should be matter of concern. In fact in patients with early stages of CHF the inflammatory process may be adaptive and protective (i.e. in ischaemic heart disease), whereas in severe CHF the chronic inflammation promotes the progress of disease. The complete antagonism of TNF-α could be harmful with in regard to the potential cytoprotective effect by the endogenous physiological levels. In addition the intrinsic toxicity of the used agents could have played a substantial role. Etanercept is able to increase TNF-α levels temporarily at high amounts via its pharmacodynamic process and therefore acts as a partial agonist (39). This increase of TNF-α bioactivity might not be problematic in rheumatoid arthritis wherein TNF-α is encapsulated within a joint space and peripheral circulating levels are relatively low, while in sepsis etanercept led to worsening clinical outcomes.

The Infliximab used in the ATTACH study reached unexpected high circulating levels. In addition intrinsic toxicity may have played a major role. Thus Infliximab exerts its effects at least in part by fixing complement in cells that express TNF on membranes. Accordingly membrane bound TNF may lead to myocyte lysis by membrane attack complex (40). This side effect appears to be beneficial in Crohns disease (elimination of activated T-cells), whereas it is very likely to be deleterious in failing myocytes expressing TNF on their surface.

On the other hand the addition of anti TNF agents on top of the current accepted therapy in CHF may have no or even adverse effects. Notably ACE-inhibitors and angiotensin receptor antagonists do depress TNF in the heart (41). Nevertheless, it is also conceivable that the concept of selective blocking of one important pro-inflammatory cytokine such as TNF-α may be fundamentally flawed. A more general inhibition of pro-inflammatory activity may be mandatory. In this respect, it is remarkable that regular physical exercise can result in a more general attenuation of the pro-inflammatory state in patients with heart failure.

Physical exercise and inflammation in heart failure. a therapeutic option?

Numerous studies have demonstrated the beneficial effect of endurance training in patients with CHF leading to improvement of peak exercise capacity (42). While submaximal cardiac output remains unchanged, physical training improves exercise tolerance, associated with decreased peripheral vascular resistance and reversal of endothelial dysfunction (43, 44). Periodic increase in shear stress of blood flow and reduction in the activity of catecholamines cause an increase in the expression of iNOS with enhanced endothelial release of nitric oxide.

More recently several studies have shown that exercise training in heart failure is associated with attenuation of the systemic pro-inflammatory state. Physical training reduces markers of inflammation in patients with heart failure such as GM-CSF, MCP-1, sICAM, sVCAM (45); TNF-α and TNF receptors (46, 47) and sFAS (48). In fact, in some of these studies a correlation was found between the improvement in exercise capacity and the training-induced reduction of plasma levels of TNF-α or sFAS-ligand in patients with chronic heart failure following a prolonged training programme (49). The change in circulating cytokines may also

depend on the type of exercise training applied, i.e. significant reduction of TNF receptor 2 was observed following bicycle training but not after electrical muscle stimulation (50). Importantly, physical training significantly reduced local expression of TNFalpha, IL-6, IL1ß and iNOS in skeletal muscle of patients with chronic heart failure whereas circulating levels of cytokines were not significantly affected in this study(51), suggesting that exercise training in heart failure reduces skeletal muscle inflammation. However, this avidensed is observational and does not prove a cause-effect relationship. Nevertheless, these observations need to be viewed in the context of other findings suggesting that exercise training in heart failure enhances the antioxidative capacity of skeletal muscle in patients with heart failure. In this respect, exercise training has been shown to increase the expression of anti-oxidative enzymes such as SOD and the glutathione system (52). These findings are reminiscent of a large body of evidence that exercise training in normal individuals increases skeletal muscle antioxidant capacity which in turn, may improve performance and delay muscle fatigue (53). In fact, it has been suggested that the health-related effects of physical fitness are, in part, related by an anti-inflammatory mechanism (54). It is interesting to note that growth hormone administration in patients with heart failure is associated with reductions of pro-inflammatory cytokines and soluble Fas ligand and may improve the clinical performance and exercise capacity in these patients (55).

One may speculate that one of the beneficial effects of exercise training in heart failure may be to increase the capacity of skeletal muscle to scavenge exercise-induced production of reactive oxygen species (ROS). The inactivation of exercise-related ROS would decrease ROS levels in skeletal muscle, reduce inflammation, decrease skeletal muscle catabolism and increase force generation. Thus, physical exercise may be able to effectively break the vicious circle of neurohormonal and immuno-inflammatory stimulation of skeletal and respiratory muscle and so improve exercise capacity and quality of life in patients with CHF. This mechanism of action may be one potential explanation for the encouraging results of a collaborative meta-analysis which shows a clear evidence that exercise training in CHF patients is safe and reduces mortality (56). Taken together, it appears that exercise training represents an effective means in patients with CHF of modulating the inflammatory state.

References

1. Holzinger C, Schollhammer A, Imhof M, et al. Phenotypic patterns of mononuclear cells in dilated cardiomyopathy. Circulation 1995;92:2876-85.
2. Patella V, Marino I, Arbustini E, et al. Stem cell factor in mast cells and increased mast cell density in idiopathic and ischemic cardiomyopathy. Circulation 1998;97:971-8.
3. Hara M, Ono K, Hwang MW et al. Evidence for a role of mast cells in the evolution to congestive heart failure. J Exp Med 2002;195:375-81.
4. Niebauer J, Volk HD, Kemp M et al. Endotoxin and immune activation in chronic heart failure: a prospective cohort study. Lancet. 1999; 353: 1838-42
5. Torre-Amione G, Kapadia S, Benedict C et al. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD). J Am Coll Cardiol 1996;27:1201-6.

6. Rauchhaus M, Doehner W, Francis DP, et al. Plasma cytokine parameters and mortality in patients with chronic heart failure. Circulation 2000;102:3060-7.
7. Benedict CR, Weiner DH, Johnstone DE, et al. Comparative neurohormonal responses in patients with preserved and impaired left ventricular ejection fraction: results of the Studies of Left Ventricular Dysfunction (SOLVD) Registry. The SOLVD Investigators. J Am Coll Cardiol 1993;22:146A-153A.
8. Bozkurt B, Kribbs SB, Clubb FJ Jr, et al. Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats. Circulation 1998;97:1382-91.
9. Franco F, Thomas GD, Giroir B, et al. Magnetic resonance imaging and invasive evaluation of development of heart failure in transgenic mice with myocardial expression of tumor necrosis factor-alpha. Circulation 1999;99(3):448-54.
10. Kurrelmeyer KM, Michael LH, Baumgarten G, et al. Endogenous tumor necrosis factor protects the adult cardiac myocyte against ischemic-induced apoptosis in a murine model of acute myocardial infarction. Proc Natl Acad Sci U S A 2000; 97:5456-61.
11. Tsutamoto T, Hisanaga T, Wada A, et al. Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure. J Am Coll Cardiol 1998;31(2):391-8.
12. Vasan RS, Sullivan LM, Roubenoff R, et al. Inflammatory markers and risk of heart failure in elderly subjects without prior myocardial infarction: the Framingham Heart Study. Circulation 2003;107:1486-91.
13. Fuchs M, Hilfiker A, Kaminski K et al. Role of interleukin-6 for left ventricular remodeling and survival after experimental myocardial infarction. FASEB J 2003;17:2118-20.
14. Podewski EK, Hilfiker-Kleiner D, Hilfiker A, et al. Alterations in Janus kinase (JAK)-signal transducers and activators of transcription (STAT) signaling in patients with end-stage dilated cardiomyopathy. Circulation 2003;107:798-802.
15. Hilfiker-Kleiner D, Hilfiker A, Fuchs M, et al. Signal transducer and activator of transcription 3 is required for myocardial capillary growth, control of interstitial matrix deposition, and heart protection from ischemic injury. Circ Res 2004;95:187-95.
16. Drexler H, Roede U, Munzel T et al. Alterations of skeletal muscle in chronic heart failure. Circulation. 1992; 85: 1751-1759
17. Pina IL, Apstein CS, Balady GJ et al. Exercise and heart failure: A statement from the American Heart Association Committee on exercise, rehabilitation, and prevention. Circulation. 2003; 107: 1210-25.
18. Drexler H. Endothelium as a therapeutic target in heart failure. Circulation. 1998; 98: 2652-5.
19. Tracey KJ, Wei H, Manogue KR et al. Cachectin/TNF induces cachexia, anemia and inflammation. J Exp Med 1988; 167: 1211-1227.
20. Patiag D, Gray S, Idris I et al. Effects of TNFalpha and inhibition of protein kinase C on glucose uptake in L6 myoblasts. Clin Sci (Lond) 2000; 99: 303-307.
21. Meadows KA, Holly JM, Stewart CE. TNF-alpha induced apoptosis is associated with suppression of insulin-like growth factor binding protein-5 secretion in differentiating murine skeletal myoblasts. J Cell Physiol 2000; 183: 330-337.
22. Li Li YP, Schwartz RJ, Waddell ID et al. Skeletal muscle myocytes undergo protein loss and reactive-oxygen-mediated NF-kappaB activation in response to TNF-alpha. FASEB J 1998; 12: 871-880

23. Riede UN, Forstermann U, Drexler H. Inducible nitric oxide synthase in skeletal muscle of patients with heart failure. J AM Coll Cardiol. 1998; 32: 964-969.
24. Hambrecht R, Adams V, Gielen S et al. Exercise intolerance in patients with chronic heart failure and increased expression of inducible nitric oxide synthase in the skeletal muscle. J AM Coll Cardiol. 1999; 33: 174-179
25. Buck M, Chojkier M: Muscle wasting and differentiation induced by oxidative stress in a murine model of cachexia is prevented by inhibitors of nitric oxide synthesis and antioxidants. EMBO J 1996; 15: 1753-1765
26. Adams V, Jiang H, Yu J et al. Apoptosis in skeletal myocytes of patients with chronic heart failure is associated with exercise intolerance. J AM Coll Cardiol. 1999; 33: 959-956
27. Vescovo G, Volterrani M, Zennaro R et al: Apoptosis in the skeletal muscle of patients with heart failure: investigation of clinical and biochemical changes. Heart 2000; 84: 431-437.
28. Dalla Libera L, Sabbadini R, Renken C et al. Apoptosis in the skeletal muscle of rats with heart failure is associated with increased serum levels of TNF-alpha and sphingosine. J Mol Cell Cardiol 2001; 33: 1871-1878
29. Libera LD, Zennaro R, Sandri M et al. Apoptosis and atrophy in rat slow skeletal muscles in chronic heart failure. Am J Physiol 1999; 277: C982-986
30. Wilcox P, Osborne S, Bressler B. Monocyte inflammatory mediators impair in vitro hamster diaphragm contractility. Am Rev Respir Dis. 1992; 146: 462-464
31. Wilcox P, Wakai Y, Walley KR et al. TNF-alpha decreases in vivo diaphragm contractility in dogs. Am J Resp Crit Care Med 1994; 150: 1368-1373
32. Li X, Moody MR, Engel D et al. Cardiac-specific overexpression of TNF-alpha causes oxidative stress and contractile dysfunction in mouse diaphragm. Circulation 2000. 102: 1690-1696
33. Murrant CL, Andrade FH, Reid MB. Exogenous reactive oxygen and nitric oxide alter intracellular oxidant status of skeletal muscle fibers. Acta Physiol Scand 1999; 166: 111-21
34. Agoston I, Dibbs ZI, Wang F, et al. Preclinical and clinical assessment of the safety and potential efficacy of thalidomide in heart failure. J Card Fail 2002;8:306-14.
35. Sliwa K, Skudicky D, Candy G et al. Randomised investigation of effects of pentoxifylline on left-ventricular performance in idiopathic dilated cardiomyopathy. Lancet 1998; 351: 1091-93.
36. Bozkurt B, Torre-Amione G, Warren MS, et al. Results of targeted anti-tumor necrosis factor therapy with etanercept (ENBREL) in patients with advanced heart failure. Circulation 2001;103:1044-7.
37. Mann D, McMurray JJ, Packer M et al. Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). Circulation. 2004; 109: 1594-602
38. Chung ES, Packer M, Lo KH, et al. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation 2003;107:3133-40.
39. Klein B, Brailly H. Cytokine-binding proteins: stimulating antagonists. Immunol Today 1995;16:216-20.
40. Homeister JW, Lucchesi BR. Complement activation and inhibition in myocardial ischemia and reperfusion injury. Annu Rev Pharmacol Toxicol 1994;34:17-40.

41. Flesch M, Hoper A, Dell'Italia L et al. Activation and functional significance of the renin-angiotensin system in mice with cardiac restricted overexpression of tumor necrosis factor. Circulation. 2003;108:598-604
42. Afzal A, Brawner C, Keteyian J. Exercise training in heart failure. Progress in Cardiovascular Diseases 1998; 41: 175-190
43. Hambrecht R, Fiehn E, Weigl C et al. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. Circulation. 1998; 98: 2709-2715
44. Hornig B, Maier V, Drexler H. Physical training improves endothelial function in patients with chronic heart failure. Circulation. 1996; 93:210-4.
45. Adamopoulos S, Parissis J, Kroupis C, et al. Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. European Heart Journal 2001; 22: 791-797
46. Larsen AI, Aukrust P, Aarsland T, et al. Effect of Aerobic Exercise Training on Plasma Levels of Tumor Necrosis Factor Alpha in Patients With Heart Failure. The American Journal of Cardiology 2001; 88: 805-808
47. Conraads VM, Beckers P, Bosmanns J, et al. Combined endurance/resistance training reduces plasma TNF-a receptor levels in patient with chronic heart failure and coronary artery disease. European Heart Journal 2002; 23: 1854-1860
48. Adamopoulos S, Parissis J, Karatzas D, et al. Physical Training Modulates Proinflammatory Cytokines and the Soluble Fas/Soluble Fas Ligand System in Patients With Chronic Heart Failure. J Am Coll Cardiol. 2002; 39: 653-63
49. Adamopoulos S, Parissis J, Paraskevaidis I, et al. Effects of growth hormone on circulating cytokine network, and left ventricular contractile performance and geometry in patients with idiopathic dilated cardiomyopathy. European Heart Journal 2003; 24: 2186-2196
50. LeMaitre JP, Harris S, Fox KAA, et al. Change in circulating cytokines after 2 forms of exercise training in chronic stable heart failure. American Heart Journal 2004;147: 100-5
51. Gielen S, Adams V, Möbius-Winkler S, et al. Anti-inflammatory effects of exercise training in the skeletal muscle of patients with chrinic heart failure. J Am Coll Cardiol. 2003; 42: 861-8
52. Ennezat PV, Malendowicz SL, Testa M et al. Physical training in patients with chronic heart failure enhances the expression of genes encoding antioxidative enzymes. J Am Coll Cardiol. 2001; 38:194-8
53. Powers SK, Li LJ, Leeuwenburgh C. Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. Med. Sci. Sports Exerc. 1999; 31: 987-997
54. Aronson D, Sheikh-Ahmad M, Avizohar O et al. C-Reactive protein is inversely related to physical fitness in middle-aged subjects. Arteriosclerosis 2004; 176: 173-179
55. Adamopoulos S, Parissis JT, Georgiadis M. Growth hormone administration reduces circulating proinflammatory cytokines and soluble Fas/ soluble Fas ligand system in patients with chronic heart failure secondary to idiopathic dilated cardiomyopathy. Am Heart J 2002; 144: 559-64
56. ExTraMATCH Collaborative. Exercise training meta-analysis of trials in patients with chronic heart failure (ExTraMATCH) BMJ 2004, 1-7