Apoptosis and changes in contractile protein pattern in the skeletal muscle in heart failure

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ABSTRACT

Chronic heart failure is characterized as a clinical disorder by exercise intolerance. There are two factors that are independently responsible for the reduced exercise capacity: (a) a shift from myosin heavy chain 1 (MHC1) to MHC2a and MHC2b and (b) muscle atrophy. We have demonstrated, both in experimental models of heart failure and in man, that the more severe the heart failure, the greater the magnitude of skeletal muscle apoptosis. In the monocrotaline treated rat, that develops a severe right-sided heart failure, the increased number of apoptotic nuclei was paralleled by increasing levels of circulating TNFα. In agreement with some recent observations showing that sphingolipids can mediate programmed cell death, we found that in animals with heart failure and high number of apoptotic nuclei, circulating levels of sphingosine were significantly increased. In a study conducted in patients with heart failure we found a correlation between exercise capacity limitation and skeletal myocytes apoptosis. There was also a correlation between degree of muscle atrophy and magnitude of apoptosis. The shift in MHCs, although with a different mechanism, is also responsible for the reduced exercise capacity in these patients. In fact there is a strong correlation between indices of severity of CHF and MHC composition. Muscle fatigue, appears earlier in patients that have a greater skeletal muscle expression of ‘fast’ MHCs. We have also demonstrated that MHCs shift and apoptosis can be prevented by using angiotensin II converting enzyme inhibitors and angiotensin II receptor blockers.

Keywords angiotensin II, apoptosis, chronic heart failure, myosin heavy chains, skeletal muscle, sphingosine, TNFs.

ORIGIN OF SYMPTOMS IN CHRONIC HEART FAILURE: THE MUSCLE HYPOTHESIS

Chronic heart failure (CHF) is characterized as a clinical disorder by exercise intolerance. The symptom causing a subject to stop exercising is usually given by shortness of breath or fatigue. During exercise, there is an early and prolonged release of lactate from exercising muscle in patients with CHF, even during light exercise (Reddy et al. 1988). The concept of lactate threshold corresponding with a measurable ventilatory anaerobic threshold has become widespread, and has consistently been found to occur at a lower level of exercise in CHF (Weber et al. 1982, Hoh et al. 1990). At first sight, fatigue may be thought of simply as a result of failure of perfusion of the exercising musculature and the consequent early onset of intramuscular acidosis; however, evidence increasingly points to there being intrinsic abnormalities of muscle metabolism and structure in patients with CHF. In fact, there is a close correlation between exercise capacity and measurements of metabolic gas exchange, particularly peak VO2. However, peak VO2 poorly correlates with indices of central haemodynamic function (Fink et al. 1986, Sullivan et al. 1989) and with measurements of peripheral blood flow as suggested by Wilson et al. (1984). These latter showed in fact that exertional fatigue is as a result of skeletal muscle dysfunction rather than to reduced skeletal muscle blood flow. For these reasons investigations have been carried out looking at skeletal muscle abnormalities. Changes in histology (Lipkin et al. 1988), mitochondria (Drexler 1992), oxidative enzyme activity (Sullivan et al. 1990)

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and high energy phosphate handling (Massie et al. 1987) together with sympathetic overactivity and parasympathetic withdrawal (Adamopoulos et al. 1993) have been described. There are some other evidences supporting the theory that central haemodynamics do not determine exercise tolerance in CHF. Drug therapy, including angiotensin converting enzyme (ACE) inhibitors, improve rapidly central haemodynamic, while increases in exercise capacity are delayed for weeks and months (Wilson et al. 1993). Pulmonary capillary wedge pressure (Sullivan et al. 1989) does not correlate well with exertional breathlessness, suggesting a crucial missing link in the mechanisms of exercise limitation in CHF. A neural linkage between ergoreceptors (afferents sensitive to skeletal muscle work) and responses to exercise has been demonstrated in CHF (Piepoli et al. 1996), their overactivation determines higher ventilation, blood pressure response and leg vasoconstriction.

There is a strong suggestion that changes in the leg skeletal muscle can limit exercise capacity and therefore be responsible for symptoms in CHF. Lipkin et al. (1988) observed in the quadriceps femoris of patients with CHF a greater expression of fast type II fibres. A range of histological abnormalities was also found with increased muscle fibre size variance, presence of atrophic fibres and intracellular lipid droplets. Later on, Mancini et al. (1989) studied biopsies of the gastrocnemius quantitatively. Although they did not find a reduction of type I fibres, a shift from IIa to IIb was reported. The type II fibres appeared to be increased in number, but decreased in size. Sullivan et al. (1990) in the vastus lateralis found type I to be decreased and type II to be increased. Capillary density was also reduced as well as enzymes of the Krebs’ cycle. They extended the study to biopsy taken during submaximal and maximal exercise (Sullivan et al. 1991) showing lactate accumulation, creatine phosphate depletion, acceleration of glycolysis and glycogenolysis at peak exercise. Nuclear magnetic resonance studies show early acidosis, drop in ATP and lactate generation (Mancini et al. 1988, 1989, Marie et al. 1990). Drexler (1992) examined the contribution of mitochondrial abnormalities to the CHF myopathy and they found a reduction of mitochondria volume and surface density of cristae. There was also a reduction in cytochrome oxidase activity. Broqvist et al. (1994) reported in the quadriceps femoris of patients with CHF a reduction of high energy metabolic substrates (ATP, glycogen and creatine phosphate). The net result of the abnormalities of lower limb skeletal muscle and blood flow appears to be the early onset of intramuscular acidosis and excessive lactate production. Skeletal muscle has been advocated as a possible determinant of symptoms in CHF via a neural linkage between peripheral abnormalities and the exaggerated exercise responses, such as the ventilatory one in heart failure (Piepoli et al. 1996). Some authors have proposed that the metabolic state of the skeletal muscle is centrally monitored by the activation of ergoreceptors. They have suggested that signals coming from these receptors are carried by fibres, travelling in the lateral spino-talamic tract, that increase ventilation and sympathetic outflow, producing vasoconstriction in distant non-exercising vascular beds, with consequent effects on blood pressure and possibly a small increase in heart rate (Coats et al. 1994). They are sensitive to the metabolic state of the muscle, but their triggers are still unclear. They have the properties necessary to link the skeletal muscle abnormality to the fatigue, dyspnea, hyperpnea and sympathoexitation characteristic of CHF. The muscle hypothesis proposes another cycle of deterioration similar to those of neuroendocrine activation. The reduction in left ventricular function determines a series of not better defined metabolic abnormalities in the skeletal muscle that have been proposed to trigger an exaggerated ergoreflex activation that is perceived by the patient as fatigue and dyspnea and leads reflex to vasoconstriction in the non-exercising muscles and excessive ventilatory response (Clark et al. 1995).

**EXERCISE CAPACITY AND MUSCLE ATROPHY**

Lipkin et al. (1988) originally showed that quadriceps femoris muscle strength is reduced and there is indeed a correlation between quadriceps strength and exercise performance as assessed by peak $V'\text{O}_2$ (Buller et al. 1991). This is because muscle mass is reduced in CHF and the more severe the limitation in exercise capacity the greater muscle bulk loss. In fact, Minotti et al. (1993) showed that there is no reduction in mean force per unit area, implying that myofibril force production was normal, but that loss of skeletal muscle mass is an important determinant of muscle strength. Mancini et al. (1992) showed that muscle wasting occurred in even mild heart failure. On the other hand, exercise capacity correlates well with both strength and mass and it was proposed that reduced leg blood flow may be a consequence of muscle wasting (Volterrani et al. 1994). Moreover, there is no relationship between exercise capacity and blood flow measurements either at rest or following exercise and ischaemia. They concluded that flow per unit muscle bulk is not a determinant of exercise capacity. Recently it has been shown that in cardiac cachexia, which is characterized by an extreme degree of muscle waste, the relationship between muscle mass, exercise capacity and peak $V'\text{O}_2$ is lost (Anker et al. 1997). Even the strength per unit area is decreased because of the severe alterations in muscle fibres and their replacement with interstitial tissue.
CHANGES IN SKELETAL MUSCLE MYOSIN HEAVY CHAIN COMPOSITION IN CHRONIC HEART FAILURE

One method for defining skeletal muscle fibres type is through their myosin heavy chain (MHC) pattern. There are three major MHCs that can be separated electrophoretically on the basis of their relative mobility. Type I fibres are mainly composed by MHC1, typical of fatigue resistant fibres, and are characterized by low ATP consumption and low speed of shortening. Fibres type IIa and IIb are mainly constituted by MHC2a and MHC2b, respectively, the fast isoforms. They possess higher ATP consumption, higher speed of shortening and are both more fatigable. We have recently shown that in the g trocneumii of patients with CHF there is a shift from the slow to the fast isoforms (Vescovo et al. 1996). The magnitude of this shift correlates with indexes of severity of CHF syndrome such as New York Heart Association (NYHA) class, exercise test tolerance measured in minutes, diuretic consumption and ejection fraction, although the relationship with this latter parameter was much weaker. There was, however, no relationship with ventricular diameters. Similar observations were made at the same time by Sullivan et al. (1997) in the vastus lateralis. The CHF skeletal muscle myopathy is generalized and involves diaphragmatic muscle (Lindsay et al. 1992, Tikunov et al. 1997). Skeletal muscle MHCs composition is partially responsible for the reduced exercise capacity in patients with CHF. In fact there is a strong positive correlation between MHC1 and peak VO2, ventilatory threshold (VT) and O2 pulse (Vescovo et al. 1998b), while there is a negative correlation between MHC2a, MHC2b and the same cardiopulmonary parameters. It is therefore possible that a high percentage of glycolitic fibres reduces exercise capacity because of the early appearance of anaerobic metabolism, as a result of the prevalence of fast fatigable fibres with low anaerobic threshold and high ATP consumption. Improvement in exercise tolerance can be obtained after endurance training (Belardinelli et al. 1995, Magnusson et al. 1996) or pharmacological treatment (Schaufelberger et al. 1996) and is accompanied by favourable histologie, metabolic and functional parameters occurring in the leg skeletal muscle. We have recently demonstrated (Vescovo et al. 1998a) that 6 months treatment with either Losartan [an angiotensinII (AngII) type 1 receptor blocker] or Enalapril (an ACE inhibitor) in patients with CHF was able to improve exercise capacity, with a significant increase in MHC1. There were no changes in muscle mass as measured by the g trocneumii computerized tomography scan cross-sectional area. As we did not find any correlation between MHC composition and neither force generation, nor muscle mass (Vescovo et al. 1998a) we can speculate that the improved exercise capacity is related to ‘fibres fatigability’ rather than to muscle strength or force generated. The observation that muscle strength depends on muscle mass and that there is no correlation between fibres type, and therefore MHC composition, confirm the hypothesis that the CHF myopathy is specific and not related to muscle atrophy.

IS APOPTOSIS THE CAUSE OF SKELETAL MUSCLE ATROPHY?

The debate on the possible causes of muscle atrophy is still open. It has been hypothesized that cytokine activation and loss of anabolic function (McMurray et al. 1991), ergometaboloreceptors dysfunction (Coats et al. 1994) or changes in blood flow (Wilson et al. 1984, Massie et al. 1987) may be of importance. We have recently found in the tibialis anterior and in the soleus of rats with CHF (Vescovo et al. 1998c) an increased number of apoptotic nuclei, during the development of CHF. In fact in both muscles the terminal transferase dUTP nick-end labelling (TUNEL) positivity was much greater in animals with heart failure as compared with that of controls. The interstitial nuclei, distinguished from the myonuclei on the basis of laminin staining, showed a much higher magnitude of apoptosis. Although apoptosis was present both in fast and slow fibres (stained with antibodies against MHC1 and MHC2b), fast muscles are more prone to develop it than slow muscles, which contain more slow-twitch fibres (0.062 ± 0.03% in the soleus vs. 0.19 ± 0.055 in the tibialis anterior). Similarly, fast muscles develop atrophy much earlier than slow muscles. Muscle fibres cross-sectional area, which is a good index of atrophy, is smaller in fast than in slow muscles (Dalla Libera et al. 1999). The pro-apoptotic factor caspase-3 was significantly increased, while Bcl-2, that is protective against apoptosis, dropped significantly (Vescovo et al. 1998c). Atrophy is preceded by the shift toward the fast isoforms once again confirming that biochemical changes are independent from muscle waste. Apoptosis in the skeletal muscle appears at the same time that CHF worsen and is paralleled by increased levels of circulating TNFz. This cytokine is known to produce muscle waste either by triggering apoptosis or by activating ubiquitin, which in turn induces protein-waste (Krown et al. 1996). In our model the ubiquitin pathway seems not to be involved, in that tissue levels of this molecule are not increased in the muscle of animals with CHF (Llovera et al. 1997). Therefore, we think that muscle atrophy is apoptosis-dependent. We have also found a similar result in humans. By studying biopsies of the vastus lateralis of patients with severe heart failure...
we have shown the presence in this muscle of atrophy and apoptosis (Vescovo et al. 2000). A similar observation has been made by Adams et al. (1999) who showed in patients with CHF increased levels of skeletal muscle apoptosis. Apoptosis was accompanied by a higher tissue expression of inducible nitric oxide synthase (iNOS) (Hambrecht et al. 1999) and was more pronounced in patients who have lower peak VO\textsubscript{2}.

Our data, although collected in a small series of patients, come from surgical biopsies that allow a precise quantitation of the phenomenon. We showed that there is a strong inverse correlation between the number of apoptotic myonuclei and the degree of muscle atrophy as measured by the fibres cross-sectional area (Vescovo et al. 2000) (Fig. 1). We also found a significant correlation between peak VO\textsubscript{2} and number of TUNEL positive cells which indicate that the more depressed exercise capacity, the more pronounced apoptosis. The degree of fibres atrophy (measured as fibres cross-sectional area) correlates with muscle endurance (% decrease in muscle strength during a repetitive exercise), and exercise capacity (peak VO\textsubscript{2}) suggesting that muscle strength depends on mass and is in turn a determinant of exercise capacity (EC). However, we could not show any correlation between degree of atrophy and MHCs composition. Skeletal muscle MHCs composition is in fact thought to influence EC because of its intrinsic characteristic fatigability. We can speculate that interstitial nuclei are mainly those of endothelial cells, which undergo apoptosis in CHF together, and to an even higher degree, with myofibres nuclei once more suggesting that apoptosis and atrophy are tightly linked (Vescovo et al. 1998c, Dalla Libera et al. 1999). We have hypothesized that endothelial apoptosis, even in the absence of changes in skeletal muscle blood flow, could alter myofibres nutrition and induce relative ischaemia to which muscle fibres adapt by shifting toward the anaerobic isoforms.

Apoptosis is also known to be involved in myocyte loss both in CHF (Olivetti et al. 1997) and in cardiomyopathies (Narula et al. 1996). It has been suggested that in the heart, apoptosis can be triggered by activation of cytokines, such as TNF-α (Krown et al. 1996) or by hormones, such as AngII (Kajstura et al. 1997). However, it is not clear whether AngII determines apoptosis via the AngII type 2 receptor (AT\textsubscript{2}) or AngII type 1 receptor (AT\textsubscript{1}) stimulation. Many authors have in fact suggested that AT\textsubscript{2} receptor stimulation brings about apoptosis (Yamada et al. 1996, Li et al. 1998, Chevalier et al. 1999, Fortuno et al. 1999, Lehtonen et al. 1999). On the other hand, Leri et al. (1998), Fortuno et al. (1998) and Li et al. (1997) have shown that Losartan, an AT\textsubscript{1} receptor blocker, and Captopril, an ACE-inhibitor, can block apoptosis both in vitro (isolated stretched myocytes) and in vivo (spontaneously hypertensive rats with CHF).

All these results are intriguing and we have recently tested the hypothesis that blocking the AT\textsubscript{1} receptor with Irbesartan can prevent apoptosis and therefore influence skeletal muscle bulk loss.

We have shown in the monocrotaline-treated rat with CHF that Irbesartan can protect from the development of skeletal myocyte apoptosis and therefore from muscle atrophy, as demonstrated by the occurrence of a significantly lower number of TUNEL-positive cells, by lower levels of Caspase-3, by increased levels of Bcl-2, and by an increased fibres cross-sectional area (L. Dalla Libera, R. Zennaro, M. Sandri, G.B. Ambrosio & G. Vescovo, unpublished observation). At the same time the MHCs pattern in the Irbesartan group was similar to that of controls. These changes seem to be independent from the severity of haemodynamic decompensation as Irbesartan was able to modify only partially the severity of hypertrophy and failure. Circulating levels of TNF-α were also decreased in the Irbesartan group, suggesting once again that apoptosis could be reduced by blocking TNF-α. We have also shown that apoptosis is accompanied by an increased level of circulating sphingosine (a phospholipid which has been shown by Krown et al. (1996) to be the second messenger of TNF-α in the heart). This second messenger were decreased to the level of control animals after Irbesartan treatment. We have postulated that AngII receptor blockade could protect from the development of skeletal muscle

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**Figure 1** Correlation between number of apoptotic nuclei (nuclei mm\textsuperscript{−1}) and myofibres cross-sectional area (μ\textsuperscript{2}) in nine patients with different degrees of CHF. The cross-sectional area represents the mean area calculated on roughly 400 fibres from a biopsy taken from the vastus lateralis. The area was calculated with a computerized planimeter. \( r^2 = 0.59, 0.51 \) and 0.58, \( P = 0.0014, 0.004 \) and 0.0015 for total, interstitial and myocyte nuclei, respectively. The apoptotic nuclei were distinguished, on the basis of a double staining with TUNEL and laminin, in myocyte, interstitial and total (which is the sum of myocyte and interstitial).
apoptosis-dependent atrophy and from the detrimental changes in fibres type composition. Whether this is because of the block of the AngII subtype 1 receptor or to the lower severity of haemodynamic decompensation cannot be said and needs further investigation.

REFERENCES


Skeletal muscle apoptosis in heart failure · G Vescovo et al.


