Metabolic Disorders of Skeletal Muscle in Congestive Heart Failure

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Abstract

Patients with congestive heart failure (CHF) usually show a reduced maximal exercise capacity, associated with muscle fatigue and dyspnea. Fatigue has been attributed to skeletal muscle abnormalities with impairment of blood flow, myofibre changes, metabolic abnormalities and atrophy. We studied peripheral skeletal muscle metabolism in CHF secondary to pulmonary hypertension induced in rats by a single injection of monocrotaline. Oxidative metabolism expressed in terms of tissue content of creatine phosphate (CP) and purine nucleotides (ATP, ADP and AMP) and redox cellular state calculated as ratio of pyridine nucleotides (NAD/NADH) was studied. In soleus muscle (oxidative) ATP declined from 20.31 ± 2.50 in age-matched animals to 9.55 ± 0.61 μmol/gdw in monocrotaline-treated rats, whereas in the extensor digitorum longus (EDL, glycolytic) ATP was reduced from 30.92 ± 2.68 to 22.70 ± 1.54 μmol/gdw. In both muscles a shift of NAD/NADH ratio towards reduction was also observed (from 26.58 ± 3.34 to 6.95 ± 0.97 in the soleus and from 18.88 ± 3.43 to 10.37 ± 1.61 in EDL). The results indicate that: (i) a decrease in muscle high energy phosphates occurs in CHF; (ii) this is paralleled by an accumulation of reducing equivalents; (iii) these metabolic alterations are more evident in the oxidative muscle.

Key words: congestive heart failure, skeletal muscle, oxidative metabolism.

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Exertional intolerance is a major clinical problem in patients with chronic heart failure and is associated with both muscle fatigue and dispnea [37]. Usually, these symptoms are alleviated by diuretics, digitalis and vasodilators therapy, with the majority of patients experiencing complete resolution of symptoms at rest. Many of them, however, continue to complain of exertional symptoms, despite optimal diuresis and medical therapy [31, 35]. It follows that their daily life is severely limited and during maximal exercise testing they must stop the exercise early because of leg fatigue.

Exertional fatigue in heart failure was traditionally attributed to skeletal muscle underperfusion. It has been shown that leg blood flow response to bicycle exercise in patients with heart failure is significantly lower than in normal subjects [38]. Patients who exhibit improved exercise capacity after therapy with angiotensin converting enzyme (ACE) inhibitors also exhibit improved leg blood flow [7].

Blood Flow in Skeletal Muscle

The reduced capacity of patients with heart failure to proportionally increase flow to the working skeletal muscle might depend on a reduction in cardiac output coupled to an impaired arteriolar vasodilatation, as evidenced by the failure of leg vascular resistance to decrease normally during exercise [19, 30]. It has been suggested that sodium retention impairs arteriolar vasodilatation by compressing the capillary bed [40]. However, removal of excess fluid and sodium retention does not normalise arterial vasodilatation [26]. The abnormal increase in noradrenaline and angiotensin II induced by exercise in patients with heart failure could also impair vasodilatation, although blocking these system does not necessarily improve blood flow [33, 34]. Other proposed possibilities include an abnormality of the vascular endothelium, leading to a reduced production of vasodilating factor [16]; a vascular remodelling due to increased angiotensin II [13] and an impaired release of vasodilators metabolites by the skeletal muscle [37].

Whatever the cause, muscle underperfusion results in intramuscular lactic acidosis, which in turn produces muscular fatigue.

Recent observations from our [6, 14] and other laboratories [39] have shown that there is a subset of patients with heart failure who show a normal increase of skeletal muscle blood flow during exercise but develop exertional fatigue and abnormal lactic acidosis. This suggests that abnormalities other than underperfusion of the working skeletal muscle might contribute to exertional fatigue. This concept is reinforced by several other findings. The increase in cardiac output caused by vasodilators even when associated to enhanced oxygen transport to skeletal muscle, is not associated with increased exercise capacity and peak oxygen consumption in patient with chronic heart failure [14]. When oxygen delivery to type I and IIA muscle fibres, which depend on oxidative muscle, is enhanced, its utilisation is not augmented, at least not acutely [6]. Numerous experimental and human studies have shown that exercise training induces major adaptations in skeletal muscle and improves exertional fatigue, independent of skeletal muscle perfusion [1, 39].

Intrinsic Abnormalities of Skeletal Muscle in Heart Failure

These observations have prompted the hypothesis that in chronic heart failure there are intrinsic abnormalities of skeletal muscle that prevent acute improvement in peak VO_2 and lead to excessive accumulation of lactate [11, 37]. Four types of observations support this concept: 1) skeletal muscle biopsy studies have provided evidence of intrinsic skeletal muscle abnormalities; 2) anthropometric and magnetic resonance imaging studies have demonstrated skeletal muscle atrophy in the majority of patients; 3) metabolic studies have shown abnormal responses to exercise in patients with heart failure, unrelated to muscle underperfusion; 4) experimental evidences in animal models of Congestive Heart Failure (CHF) have shown alterations of the oxidative energetic metabolism. These issues will be reviewed in detail.

Findings from skeletal muscle biopsies

To determine if an abnormality of skeletal muscle could be demonstrated in patients with heart failure, several authors have taken skeletal muscle biopsies from a heterogeneous group of patients with heart failure. These demonstrated a variety of different and, in part, conflicting abnormalities [4, 10, 12, 21, 22, 28].

Histological examination of skeletal muscle revealed a variable extent of atrophy, increased interstitial cellularity, and an increase in type IIB fibres [21, 22, 28]. From the biochemical point of view, Lipkin *et al* [21] noted a variety of abnormalities but no specific pattern of changes. The activity of citrate synthase, an oxidative enzyme, was normal in six patients but decreased in three. An increase in acid phosphatase was observed in six patients. Mancini *et al* [22] found in the gastrocnemius muscle of patients with heart failure a decrease in ß-hydroxyacyl-CoA activity, a mitochondrial-based enzyme involved in fatty acid metabolism. Citrate synthase, however, was normal, suggesting normal oxidative enzyme levels. Phosphofructokinase levels were also normal, suggesting maintenance of enzymes involved in glycolysis. Drexler and colleagues [8] noted in biopsies from the *vastus lateralis* of patients with heart failure a decrease in volume and surface density of mitochondrial cristae and inner boundary membranes. In addition, they discovered an increase in triadic junction cisternae which are involved in intracellular and extracellular calcium release. Based on these data, the authors suggest that muscular fatigue in heart failure may be in part, at least, related to reduced oxidative capacity of skeletal muscle, possibly caused by Ca^{2+} overloading.

Findings from anthropometric measurements

To determine if generalised muscle atrophy is present in patients with heart failure, Mancini *et al* [25] performed anthropometric measurements in 62 patients with heart failure and compared calf muscle volume as assessed with magnetic resonance imaging of patients with heart failure to control subjects.

Using these technologies skeletal muscle atrophy was noted in more than 50% of patients. Interestingly, anthropometric measurements failed to detect reduction in fat stores in the majority of patients. This suggests that muscle atrophy is not accompanied by a generalised loss of total body weight and fat stores.

Findings from metabolic studies

The first metabolic studies were conducted using phosphorus-31 nuclear magnetic resonance (NMR), a technology that permits the non-invasive monitoring of phosphocreatine, inorganic di-(ADP) and tri-(ATP) phosphates, as well as pH in working muscle. A series of studies examined the metabolic behaviour of both forearm and calf muscles in patients with heart failure. The data obtained show a more pronounced increase in the organic phosphate/phosphocreatine ratio and a more pronounced decreased in muscle pH than in normal subjects performing comparable work load [23, 32, 39]. In addition, patients with heart failure show a delayed time constant of recovery for phosphocreatine resynthesis after exercise [24]. Interestingly, the time constant of recovery correlates better with the work slope than with the muscle volume, thus suggesting that intrinsic and metabolic alterations rather than muscle atrophy are at the basis of reduced exercise tolerance in heart failure. Furthermore, these observations fit with previous findings in patients with chronic heart failure, showing that the extent of intrinsic and ultrastructural alterations of skeletal muscle is related to exercise capacity [9].

All these metabolic abnormalities are not simply due to skeletal muscle ischaemia as they also occur in the presence of normal forearm blood flow [32]. In other studies, the arterial-venous femoral difference has been measured at rest and during exercise. By using this tech-

nique, we could demonstrate that in patients with heart failure the uptake of FFA is impaired both at rest and during exercise [6, 14]. In addition, we and others have shown that the skeletal muscle metabolism in heart failure is abnormally dependent on anaerobic glycolysis, resulting in excessive accumulation and release of lactic acid [27]. These biochemical changes could not be explained by impaired blood flow or reduced oxygen delivery alone. One metabolic change that would explain these observations is a decrease in glucose and FFA oxidation relative to glycolysis. Such an imbalance would accelerate tissue acidosis during exercise.

Skeletal muscle oxidative metabolism in experimental heart failure

We have experimentally approached the issue of metabolic abnormalities in skeletal muscle during CHF by studying energetic metabolism at rest in muscle biopsies obtained from an animal model of heart failure, which enabled us to distinguish stable compensated state of the disease with respect to congestive conditions leading to the syndrome of CHF. Heart failure was induced in rats by a single injection of monocrotaline, a pyrrolizidine alkaloid that causes epithelial proliferation of the small pulmonary arteries and pulmonary hypertension of varying degrees, depending on dose and individual susceptibility [17, 18]. Consequently, a percentage of the animals develops compensatory ventricular hypertrophy with no sign of heart failure. Others, in addition to ventricular hypertrophy, present a measurable accumulation of liquid into the pleural and peritoneal spaces and typical neuroendocrine and molecular alterations thus resembling the condition of CHF in humans [5, 17]. Energetic metabolism was studied by means of a HPLC method recently developed in our laboratory [3] which allows to assess, in the same sample biopsy, high energy phosphates (CP, ATP, ADP, AMP) as well as pyridine nucleotide redox state (NAD/NADH couple).

These experimental conditions enabled us to investigate if alterations occur in oxidative capacity in skeletal muscles of rats with varying severity of heart failure.

The changes in energetic metabolism of treated rats were compared with those of two distinct control groups. There is in fact an age difference (3 weeks) between the rats with early onset of CHF and those with the chronic compensatory phase of the disease. The decreased energetic level of CP, ATP and energy charge (EC) determined in 3 weeks-older rats required to add an older control group. These differences can be ascribed to the age and are statistically significant for CP in EDL and Tibialis Anterior (TA), for ATP in Soleus and EDL, and for EC in Soleus (Table). These differences were controlled by using correctly age-matched animals.

Different metabolic adaptations occurred in skeletal muscles of CHF rats (Table). In the Soleus muscle, which is mainly a slow-twitch muscle, there was a marked decrease in high energy phosphates content, CP values being about 60% of the control (51 ± 6.7 vs. $83+10.4$; $p < 0.01$) and ATP the 47% (9.6 ± 0.6 vs. 20.3 ± 2.5; $p < 0.001$). Energy charge paralleled this decrease (0.855 ± 0.01 vs. 0.905 ± 0.002; $p < 0.001$). At the same time there was an accumulation of NADH (from 0.12 ± 0.02 to 0.32 ± 0.05, 267% increase; $P < 0.01$) with a concomitant decrease of NAD^+ (from 2.94 ± 0.36 to 1.72 ± 0.15; $P < 0.01$) leading to a drop of NAD/NADH ratio which indicates an impaired oxidative utilisation of reducing equivalents at the level of citrate synthetase. There were no major differences in AMP and ADP content of the Soleus muscle of CHF rats (Table).

No changes were observed in both high energy phosphates or pyridine nucleotides in the Soleus muscle of the compensated group (Table).

CHF also caused changes in high energy phosphates and cellular redox state of EDL muscle, which is mainly fast-twitch. The changes were similar, although less pronounced, to those observed in Soleus. CP decreased from 127 ± 12.4 to 78.7 ± 7.8 ($p < 0.01$) and ATP from 30.9 ± 2.7 to 22.7 ± 1.5 ($p < 0.05$). AMP and ADP were unchanged. The energy charge decreased slightly, from 0.924 ± 0.002 to 0.911 ± 0.005 ($p < 0.05$). NAD decreased and NADH increased yielding a ratio of 18.9 ± 3.4 significantly higher with respect to that of the age-matched control rats (10.6 ± 1.6; $p < 0.05$). There were no significant changes in the stable Compensated Group.

In TA muscle, which is a mixture between slow and fast-twitch fibres, there were no major changes in energetic metabolism both in the CHF and in the Compensated Groups.

These results provide evidence that, in CHF, high energy phosphates and energy charge of peripheral muscle may be already decreased at rest, depending on the type of muscle examined. This is evident not only in comparison with controls, but also respect to animals with heart hypertrophy adaptation to the overload and implies that profound metabolic abnormalities occur with progression of disease. These observations should also be analysed in the light of the data on pyridine nucleotides and cellular redox potential. Under any condition in which high energy phosphates were decreased, an accumulation of NADH and a shift of Redox potential towards an accumulation of reducing equivalents was observed. This is the typical metabolic situation that one would expect when oxidative utilisation of pyruvate and acetyl-CoA is rate-limited and expresses the inadequacy of oxidative-phosphorylation processes of the mitochondria.

Consequently these data imply that the decrease in high energy phosphates and energy charge in CHF is due to a decreased oxidative capacity of the resting muscle which can be due either to decreased oxygen availability or to an impairment of oxygen utilisation.

Furthermore, whatever the primary cause of the impaired oxidative capacity, it may play a role in limiting muscle function and therefore is an important feature in the patho-

Table. Tissue metabolite content in experimental congestive heart failure.

	CHF		Compensated	
	Monocrotaline treated (n = 12)	Control (n = 12)	Monocrotaline treated (n = 12)	Control (n = 12)
		Soleus		
CP	50.99 ± 6.71**	83.00 ± 10.42	70.67 ± 11.73	62.80 ± 7.85
AMP	0.35 ± 0.07	0.35 ± 0.05	0.28 ± 0.06	0.26 ± 0.03
ADP	3.05 ± 0.26	3.86 ± 0.38	4.12 ± 0.71	3.52 ± 0.28
ATP	9.55 ± 0.61+	20.31 ± 2.50	9.53 ± 3.30	15.61 ± 1.28
EC	0.855 ± 0.01+	0.905 ± 0.002	0.900 ± 0.004	0.896 ± 0.003
NAD	1.72 ± 0.15**	2.94 ± 0.36	2.86 ± 0.42	2.71 ± 0.19
NADH	0.32 ± 0.05**	0.12 ± 0.02	0.10 ± 0.01	0.12 ± 0.01
		Extensor Digitorum Longus		
CP	78.72 ± 7.79**	127.01 ± 12.36	88.15 ± 12.61	67.14 ± 6.79
AMP	0.35 ± 0.07	0.30 ± 0.04	0.25 ± 0.06	0.21 ± 0.02
ADP	4.11 ± 0.28	4.84 ± 0.40	4.14 ± 0.37	3.66 ± 0.30
ATP	22.70 ± 1.54*	30.92 ± 2.68	25.60 ± 2.64	22.13 ± 1.95
EC	0.911 ± 0.005*	0.924 ± 0.002	0.926 ± 0.004	0.920 ± 0.004
NAD	2.93 ± 0.24*	3.54 ± 0.25	3.11 ± 0.32	2.79 ± 0.22
NADH	0.35 ± 0.05	0.25 ± 0.04	.13 ± 0.02	0.26 ± 0.07
		Tibialis Anterior		
CP	71.16 ± 7.58	74.56 ± 7.90	42.63 ± 4.72	39.73 ± 3.58
AMP	0.23 ± 0.03	0.19 ± 0.02	0.19 ± 0.05	0.15 ± 0.02
ADP	3.93 ± 0.38	3.59 ± 0.31	4.59 ± 0.31	3.93 ± 0.22
ATP	22.32 ± 1.45	21.49 ± 1.27	24.56 ± 2.14	20.44 ± 1.26
EC	0.918 ± 0.01	0.920 ± 0.006	0.914 ± 0.004	0.913 ± 0.002
NAD	3.02 ± 0.17	2.89 ± 0.17	3.49 ± 0.33	2.78 ± 0.12
NADH	0.19 ± 0.04	0.13 ± 0.05	0.12 ± 0.01	0.12 ± 0.02

The data (µmol/g dry wt) are expressed as the means ± s.e. Statistical significance from respective control group at *$p < 0.05$, **$p < 0.01$ and +$p < 0.001$. CP, creatine phosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; EC, energy charge {([ATP]+0.5X[ADP])/([AMP]+[ADP]+[ATP])}; NAD and NADH, oxidized and reduced nicotinamide adenine dinucleotide phosphate.

physiology of CHF.

Other Potential Mechanisms of Skeletal Muscle Changes in Heart Failure

On the basis of these observations, it is clear that multiple factors contribute to exercise intolerance in patients with heart failure. Another important factor to mention is muscle deconditioning.

Deconditioning is likely to occur because patients with heart failure, partly following the physician's advice, restrict their own physical activity or are hospitalised and subjected to long periods of bed rest. Deconditioning, in turn, produces muscle atrophy and reduction in mitochondrial-based enzymes. Consistent with this concept, bicycle training in patients with heart failure has been shown to improve exercise tolerance by peripheral mechanisms, such as delaying the onset of anaerobic metabolism [29]. Training induces improvement of peripheral muscle metabolism and function appears to be independent of systemic adaptations.

Finally a decreased caloric and protein intake may also be a major contributor to skeletal muscle atrophy, at least in certain subsets of patients, such as those with alcoholic cardiomyopathy. Other factors may include increased oxygen free radicals activity [2], increased sympathetic

tone, elevated cortisol levels [15] or monocyte activation with increased plasma levels of tumor necrosis factor [20].

Conclusions

We are only beginning to realise the complexity of the peripheral alterations in heart failure. Further studies should provide important information on the role of cytokine, endothelial dysfunction and neuroendocrine activation. From the clinical point of view, the information available suggests that more attention should be given to detect, and eventually correct, skeletal muscle changes. For instance if deconditioning and/or malnutrition are diagnosed, appropriate interventions should be instituted and restriction of physical activity should be substituted by an appropriate exercise programme.

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