Int. J. Exp. Path. (2008), 89, 216–222
doi: 10.1111/j.1365-2613.2008.00587.x

ORIGINAL ARTICLE

Down-regulation of MyoD gene expression in rat diaphragm muscle with heart failure

Francis da Silva Lopes*,†, Robson Francisco Carvalho*,‡, Gerson Eduardo Rocha Campos*, Mario Matheus Sugizaki‡, Carlos Roberto Padovani‡, Célia Regina Nogueira‡, Antonio Carlos Cicogna‡ and Maeli Dal Pai-Silva‡

*Departamentos de Biologia Celular e Anatomia, UNICAMP, Campinas, Brasil, †Departamento de Fisioterapia, UNOESTE, Presidente Prudente, Brasil and ‡Departamentos de Morfologia, Clínica Médica e Bioestatística, UNESP, Botucatu, Brasil

INTERNATIONAL JOURNAL OF EXPERIMENTAL PATHOLOGY

Received for publication:
31 October 2007
Accepted for publication:
23 February 2008

Correspondence:
Dra. Maeli Dal Pai-Silva
Departamento de Morfologia
UNESP
Botucatu, 18618-000
São Paulo
Brazil
Tel./Fax: +55 14 3811 6264
E-mail: dpsilva@ibb.unesp.br

Summary

Diaphragm myopathy has been described in patients with heart failure (HF), with alterations in myosin heavy chains (MHC) expression. The pathways that regulate MHC expression during HF have not been described, and myogenic regulatory factors (MRFs) may be involved. The purpose of this investigation was to determine MRF mRNA expression levels in the diaphragm. Diaphragm muscle from both HF and control Wistar rats was studied when overt HF had developed, 22 days after monocrotaline administration. MyoD, myogenin and MRF4 gene expression were determined by RT-PCR and MHC isoforms by polyacrylamide gel electrophoresis. Heart failure animals presented decreased MHC IIa/IIx protein isoform and MyoD gene expression, without altering MHC I, IIb, myogenin and MRF4. Our results show that in HF, MyoD is selectively down-regulated, which might be associated with alterations in MHC IIa/IIx content. These changes are likely to contribute to the diaphragm myopathy caused by HF.

Keywords
diaphragm muscle, heart failure, myogenic regulatory factors, myosin heavy chain, Wistar rats

Heart failure (HF) is characterized by reduced tolerance to exercise because of early fatigue and dyspnoea (Wilson & Mancini 1993). Limb-muscle fatigue has been partly attributed to a shift from myosin heavy chain (MyHC) I 'slow' to MyHC II 'fast' (Simonini *et al.* 1996; Vescovo *et al.* 1998; Carvalho *et al.* 2003), whereas a shift from MyHC II to MyHC I in diaphragm muscle may contribute to dyspnoea in this syndrome (Tikunov *et al.* 1996; De Sousa *et al.* 2001). However, the skeletal muscle-specific molecular regulatory mechanisms that alter MHC expression in the diaphragm during HF are not known.

Different pathways regulate skeletal muscle MHC expression (Allen *et al.* 2001); these include myogenic regulatory factors (MRFs), a family of transcriptional factors that control the expression of several skeletal muscle-specific genes (Hughes *et al.* 1993, 1999). The family has four members: MyoD, myogenin, Myf5 and MRF4. Myogenic regulatory factors form dimers with ubiquitous E

proteins (e.g. E12 or E47) resulting in heterodimeric complexes that bind to the E-box consensus DNA sequence (5′-CANNTG-3′) found in the regulatory region of many muscle-specific genes (Murre *et al.* 1989). During embryogenesis, MRFs are critical for establishing myogenic lineage and controlling terminal differentiation of myoblasts (Parker *et al.* 2003). Previous studies have suggested that myogenin and MyoD may also be involved in establishing and maintaining slow and fast mature muscle fibre phenotype; myogenin is expressed at higher levels than MyoD in slow muscles, whereas the opposite is true for fast muscles (Hughes *et al.* 1993; Voytik *et al.* 1993). Similarly, MyoD is associated with the expression of fast type IIX and IIB MHC isoforms (Hughes *et al.* 1993, 1997; Mozdziak *et al.* 1998; Seward *et al.* 2001).

Recently in our laboratory, we have shown that mRNA relative expression of MyoD in Soleus and Extensor Digitorum Longus (EDL) muscles and that of MRF4 in Soleus muscle were significantly reduced, whereas myogenin was not changed in either muscle in Wistar rats with monocrotaline-induced HF; thus showing a potential role for MRFs in limb skeletal muscle myopathy during this syndrome (Carvalho *et al.* 2006). It would also be interesting to analyse whether MRFs gene expression in diaphragm, because of the fact that during HF, this muscle's work tends to increase both at rest and during exercise whereas limb muscle work tends to decrease (Tikunov *et al.* 1997). Therefore, this investigation was undertaken to examine MRFs mRNA and MHC protein expression in diaphragm skeletal muscle of rats with monocrotaline-induced HF.

Materials and methods

Experimental model

Fifteen weaned male Wistar rats (80–100 g) were obtained from the Central Animal House at São Paulo State University. Heart failure was experimentally induced in nine rats (HF group) by a single intra-peritoneal injection of 30 mg/kg monocrotaline, a widely accepted HF model (Vescovo *et al.* 1989, 1998; Dalla Libera *et al.* 1999, 2001, 2004; Leineweber *et al.* 2002). Monocrotaline is a pyrrolizidine alkaloid that induces pulmonary hypertension with severe right ventricle hypertrophy and failure (Vescovo *et al.* 1989; Reindel *et al.* 1990) without itself producing direct changes in skeletal muscle (Vescovo *et al.* 1998). Monocrotaline-treated rats were allowed to eat freely from a supply of standard rat chow. Six controls rats were injected with saline and fed the same amount of food as consumed by the HF rats on the previous day. Heart failure and Control groups were studied after the development of tachypnea and laboured respiration in the HF group, which occurred 22 days after monocrotaline administration. Heart failure was documented at killing by the presence of pleural and peritoneal effusions, and severe atrial and ventricular hypertrophy and dilatation. At time of the study, all animals were anaesthetized with sodium pentobarbital (50 mg/kg), and killed. Body weight (BW) was measured. The left and right portions of the costal diaphragm were isolated and immediately frozen in liquid nitrogen and stored at −80 °C. Left ventricle weight with inter-ventricular septum included (LVW) and right ventricle weight (RVW) normalized to BW (LVW/BW and RVW/BW respectively) were used as ventricular hypertrophy indexes. This experiment was approved by the Ethics Committee of the Bioscience Institute, UNESP, Botucatu, SP, Brazil.

Electrophoretic separation of MHC

Myosin heavy chains isoform analysis was performed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Six to ten serial cross sections (12 μm thick) of frozen left costal diaphragm were placed in 450 μl of a solution containing 10% (wt/vol) glycerol, 5% (v/v) 2-mercaptoethanol, 2.3% (wt/vol) SDS and 0.9% (wt/vol) Tris–HCl (pH6.8) for 10 min at 60 °C. Small quantities of the extracts (6 μl) were loaded on a 7–10% SDS-PAGE separating gel with a 4% stacking gel, run overnight (19–21 h) at 120 V and silver stained. Myosin heavy chains isoforms were identified according to molecular mass and their relative percentages were quantified by densitometry. Identification of fast and slow isoforms was accomplished by comigration of EDL muscle samples with a control animal used as pattern.

RT- PCR analysis of mRNA for MRF

Total RNA was extracted from right portions of the costal diaphragm muscles with TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), which is based on the guanidine thiocyanate method. Frozen muscles were mechanically homogenized on ice in 1 ml of ice-cold TRIzol reagent. Total RNA was solubilized in Rnase-free H_2O, incubated in Dnase I (Invitrogen Life Technologies, Carlsbad, CA, USA) to remove any DNA present in the sample, and quantified by measuring optical density (OD) at 260 nm. RNA purity was ensured by obtaining an OD ratio of approximately 2.0 at 260–280 nm. Two micrograms of RNA were reverse transcribed with random hexamer primers and Superscript II RT in a total volume of 21 μl, according to standard methods (Invitrogen Life Technologies).

Control No RT reactions were performed in which the RT enzyme was omitted. The Control No RT reactions were PCR amplified to ensure no DNA contamination of the RNA. One microlitre of cDNA was then amplified using 0.2 mM of each primer (Table 1), 1× PCR buffer minus Mg, 1.5 mM of $MgCl_2$, 0.2 mM of deoxyribonucleotide triphosphates and 1 unit of Platinum Taq DNA Polymerase (Invitrogen Life Technologies) in a final volume of 25 μl.

After reverse transcriptase reaction, the first-strand cDNA mixture was subjected to polymerase chain reaction. Target cDNA (MyoD, myogenin, MRF4 and cyclophilin) were amplified in different tubes by using aliquots from total cDNA. The number of polymerase chain reaction cycles was adjusted to avoid amplification system saturation. It is important to select the appropriate number cycles so that the amplification product is clearly visible on an agarose gel and can be quantified, but also that amplification is on exponential range and has not a plateau yet. This standard was realized to each specific PCR amplification (Figure 1). Primer pairs for MyoD were designed from sequences published in GenBank; myogenin and MRF4 primer sequences were as per Smith *et al.* (1994). Amplification products were identified by their sizes after electrophoresis on 1.0% agarose gel and stained with ethidium bromide. Images were captured and the bands corresponding to each gene were quantified by densitometry as Integrated Optical Density. The PCR products were run in duplicate on a different gel for each gene, and results averaged. The PCR products were normalized to the housekeeping gene cyclophilin (Alway *et al.* 2002; Siu *et al.* 2004).

Statistical methods

Data are expressed as mean ± standard error (SE). Comparisons were made between Control and HF groups. The Student's unpaired *t*-test was used in to analysis MRF gene expression. Myosin heavy chain isoform percentages were submitted to multivariate statistical analysis of repeated measures for two independents groups (Profile Analysis) and the Bonferroni test. Significance level was $P < 0.05$.

Results

Anatomical data

Table 2 shows anatomical data from Control and HF groups. At 22 days, all monocrotaline-treated rats showed signs of HF at postmortem examination including atrium hypertrophy, right ventricular hypertrophy (RVW/BW ≥ 0.80), ascite, pleural effusions and liver congestion. No alterations were found in control rats.

There was no significant difference in BW between HF and control groups. Heart weight was greater in HF than in control group, as demonstrated by LVW, RVW and atrium (AT) and heart hypertrophy indexes (LVW/BW, RVW/BW and ATW/BW). There was no significant difference in lung wet/dry ratio between control and HF groups. The wet RV, LV, AT and liver wet/dry ratio were greater in HF than in control group.

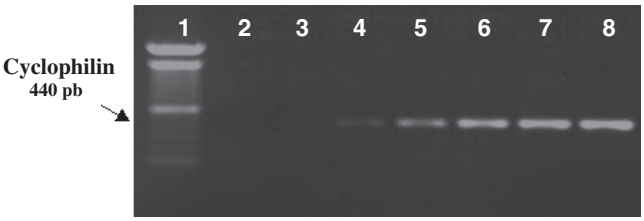

Figure 1 Representative gel of cyclophilin to determination the number cycles during PCR. Determination of number of cycles for the cyclophilin (1 = pattern molecular weight, 2 = 16 cycles; 3 = 20 cycles; 4 = 24 cycles; 5 = 28 cycles, 6 = 32 cycles; 7 = 36 cycles; 8 = 40 cycles). Product of cyclophilin PCR with 440 bp. We used 32 cycles because of the exponential phase.

Table 1. Oligonucleotide primers used in PCR amplification of reverse transcribed RNA

Product	Accession no.	Sequence	Start position	T_A (°C)	Cycles	PCR length (bp)
MyoD	NM_176079	5′-GACGGCTCTCTCTGCTCCTT	259	60	32	543
		5′-GTCTGAGTCGCCGCTGTAGT	801			
Myogenin	NM_017115	5′-TGCCACAAGCCAGACTACCCACC	828	63	31	245
		5′-CGGGGCACTCACTGTCTCTCAA	1072			
MRF-4	NM_013172	5′-AGAGACTGCCCAAGGTGGAGATTC	471	63	32	272
		5′-AAGACTGCTGGAGGCTGAGGCATC	742			
Cyclophylin	M19533	5′-ACGCCGCTGTCTCTTTTC	9	57.7	32	440
		5′-TGCCTTCTTTCACCTTCC	431			

T_A, annealing temperature.

Table 2 Experimental group anatomical data

	Experimental groups	
	Control (n = 6)	HF (n = 9)
BW (g)	158.4 ± 3.5	154.6 ± 8.4
LV wt (g)	0.37 ± 0.006	0.45 ± 0.02*
RV wt (g)	0.10 ± 0.007	0.34 ± 0.02***
AT wt (g)	0.06 ± 0.005	0.1 ± 0.01**
LV/Body wt (mg/g)	2.35 ± 0.04	2.99 ± 0.14*
RV/Body wt (mg/g)	0.65 ± 0.05	2.22 ± 0.08***
AT/Body wt (mg/g)	0.38 ± 0.03	0.65 ± 0.01*
LV w/d	4.12 ± 0.04	4.69 ± 0.054***
RV w/d	4.14 ± 0.021	4.78 ± 0.07*
AT w/d	3.40 ± 0.22	5.24 ± 0.002**
Liver w/d	3.16 ± 0.07	3.59 ± 0.036***
Lung w/d	4.83 ± 0.18	5.18 ± 0.17

Values are means ± SE.
n, number of animals; HF, heart failure group; BW, body weight; LV, left ventricle weight; RV, right ventricle weight; AT, atrium weight; and w/d, wet-to-dry weight.
*P < 0.05, **P < 0.001, ***P < 0.0001: statistical significance *vs.* control group.

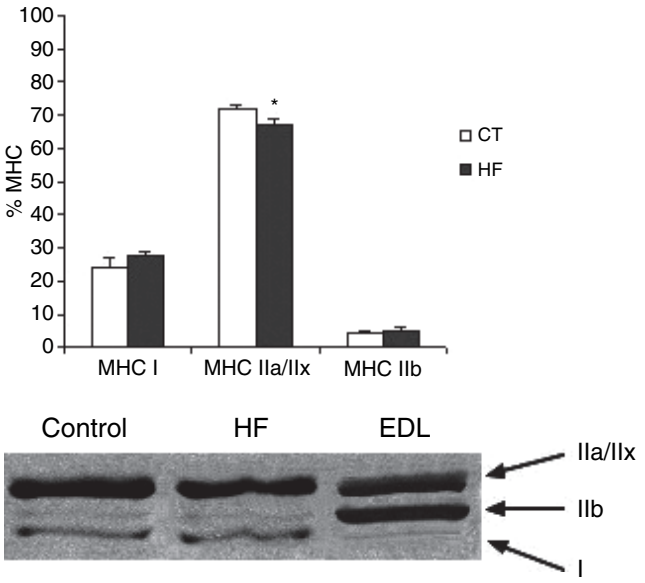

Figure 2 Percentage distribution of myosin heavy chain (MHC), MHC I, MHC IIa/IIx and MHC IIb in diaphragm muscle from control and heart failure groups. Control Extensor Digitorum Longus muscle was used as a pattern. * P < 0.05 *vs.* control group P < 0.05.

MHCs electrophoretic pattern

In diaphragm muscle, three MHC isoforms were separated, MHC I, MHC IIa/MHC IIx and MHC IIb. The difference in electrophoretic migration between MHC IIa and MHC IIx was very small, and it was not possible to quantify them separately. Consequently, we collapsed the sum of MHC IIa and MHC IIx (Bernocchi *et al.* 2003). MHC I and MHC IIb were not different between groups (CT = 23.97 ± 2.96% *vs.* HF = 27.86 ± 1.27% and CT = 4.24 ± 2.02% *vs.* HF = 4.93 ± 1.87% respectively). MHC IIa/IIx were lower in HF than in CT (CT = 71.79 ± 0.97% *vs.* HF = 67.21 ± 1.36%, P < 0.05; Figure 2).

MRF mRNA levels in diaphragm estimated by semi-quantitative RT-PCR

MyoD mRNA levels were lower in HF than in CT (CT = 2.63 ± 0.63 *vs.* HF = 0.88 ± 0.19, P < 0.05; Figure 3).

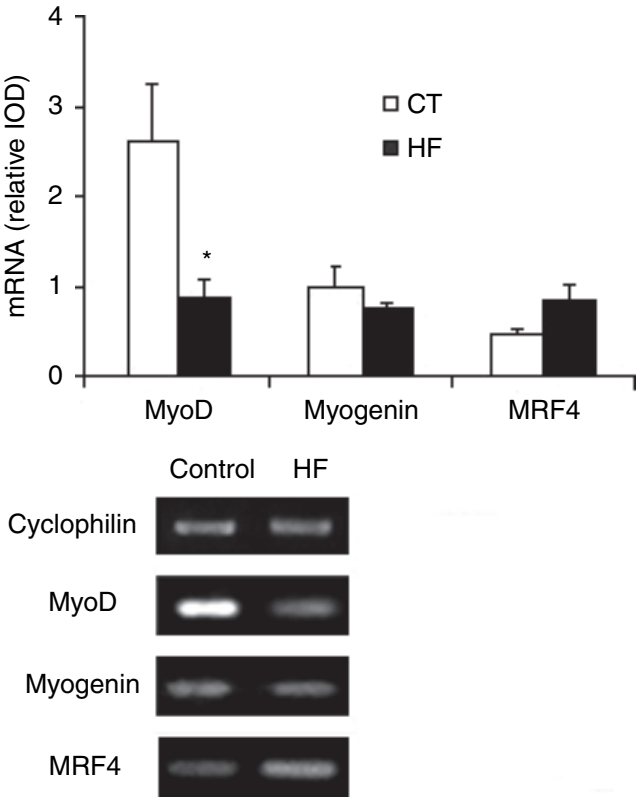

Figure 3 MyoD, myogenin and MRF4 mRNA content from rat diaphragm muscle estimated by RT-PCR in Control and heart failure groups. Samples were run in duplicate on different gels for each gene, and results averaged. PCR products were visualized with ethidium bromide staining. PCR quantification was by densitometric analysis of the product as integrated optical density. Gene expressions were normalized to cyclophilin from the same RT product. Normalized data are expressed as means ± SE.
*Data are significantly different from Controls at P < 0.05.

Myogenin expression was similar in both groups (CT = 1.0 ± 0.21 *vs.* HF = 0.74 ± 0.08). MRF4 expression was similar in both groups (CT = 0.85 ± 0.05 *vs.* HF = 0.74 ± 0.16). The MyoD to Myogenin mRNA ratio was less in HF than in CT (CT = 2.90 ± 0.79 *vs.* HF = 1.30 ± 0.31, $P < 0.05$).

Discussion

This investigation was undertaken to examine MRFs mRNA and MHC protein expression in the rat diaphragm skeletal muscle with monocrotaline-induced HF. This is the first study showing that HF decreased the relative mRNA level of MyoD without changes in mRNA relative expression of myogenin and MRF4 in the diaphragm muscle. Although during HF, diaphragm muscle work tends to increase both at rest and during exercise whereas limb muscle work tends to decrease (Tikunov *et al.* 1997), we have shown similar results in a previous study for MyoD and Myogenin mRNA relative expression in Soleus and EDL limb muscles using the monocrotaline HF model.

There is substantial evidence suggesting that MRFs are important regulators in the expression of muscle-specific proteins (Hughes *et al.* 1993; Parker *et al.* 2003). Myogenin is expressed at higher levels than MyoD in slow muscles, whereas the opposite is true for fast muscles (Hughes *et al.* 1993; Voytik *et al.* 1993). Similarly, MyoD is associated with expression of fast type IIx and IIb MHC isoforms (Hughes *et al.* 1993, 1997; Seward *et al.* 2001).

Alterations in MRF expression may be directly involved in controlling fibre type-specific gene expression in response to external signals such as hypothyroidism, chronic low-level frequency stimulation, cross-reinnervation, denervation and hindlimb suspension (Eftimie *et al.* 1991; Mozdziak *et al.* 1998; Carlsen & Gundersen 2000). In our study, the reduction in MyoD mRNA expression might be associated with the decrease in fast MHC IIa/IIx protein isoform expression seen in diaphragm muscle. The degree of decline in MyoD mRNA appeared more extensive than MHC IIa/IIx and this fact may be related to the different methods used to analyse the MRFs and MHCs.

To our knowledge, few studies have analysed the mRNA MRFs expression that might be associated with alterations in MHC protein isoform expression in diaphragm muscle during HF. Staib *et al.* (2002) showed a change in MHC protein isoform expression in diaphragm of mice MyoD −/−. MyoD deletion resulted in a significant shift from MHC IIb toward both MHC IIa and MHC IIx showing a shift in diaphragm MHC phenotype from fast to slow. Additionally, a study in diaphragm muscle from rats subjected to mechanical ventilation showed a decreased MyoD mRNA level and diminished fast MHC IIa and IIb mRNA expression; the authors suggested muscle phenotypic adaptation toward a slower profile considering the reduced MyoD/myogenin ratio, as observed in our study (Racz *et al.* 2003).

The causes of down-regulation in MyoD mRNA expression during HF are still unknown; however, neurohormones and cytokine activation may contribute (Anker *et al.* 1999). This last point has undergone considerable debate because tumour necrosis factor-alpha (TNF-α) is markedly increased in humans and animals with chronic HF (Anker *et al.* 1999; Levine *et al.* 1990; McMurray *et al.* 1991). Li *et al.* (2000) demonstrated that elevated circulating levels of TNF-α provoke contractile dysfunction in the diaphragm through an endocrine mechanism which is thought to be mediated by oxidative stress. One hallmark of TNF-α action is the activation of nuclear factor Kappa B (NFκB), a ubiquitous transcription factor normally inactive and sequestered in the cytoplasm through association with IκB (Guttridge *et al.* 2000). TNF-α exposure leads to the degradation of IκB, allowing NFκB translocation to the nucleus where it down-regulates MyoD mRNA at a posttranscriptional level (Israël 2000). On the other hand, the cooperative activity of TNF/Interferon (IFN) could reduce MyoD mRNA expression, mediated the repression of the fast MyHC promoter (Acharyya *et al.* 2004). Given that MyoD binding to the fast MyHC promoter is required for myosin expression in fast-twitch muscles (Wheeler *et al.* 1999), it is tempting to speculate that cytokine-induced suppression of fast MyHC transcription is regulated by NF-κB through the inhibited synthesis of MyoD.

This mechanism may partially explain the down-regulation of MyoD that we found in diaphragm muscle. However, further studies need to be conducted to demonstrate this mechanism during HF.

In our investigation, myogenin and MRF4 mRNA relative expression did not change during HF. It has been shown that myogenin is involved with oxidative gene expression and metabolic enzyme activity (Hughes *et al.* 1999; Ekmark *et al.* 2003; Siu *et al.* 2004), whereas MRF4 is expressed mainly after birth and is likely to have a role in skeletal muscle maintenance (Hughes *et al.* 1997). The concept that MRF4 is involved in regulating maturation events and maintaining adult skeletal muscle is further sustained by the fact that MRF4 is not expressed at substantial levels in limb until very late in foetal development (Hinterberger *et al.* 1991) and is also present at higher levels in postnatal muscle (Voytik *et al.* 1993; Hughes *et al.* 1997). Thus, both myogenin and MRF4 seem not to be involved with changes in fibre type-specific myosin heavy chain expression during HF.

In summary, our results show that in HF, MyoD is selectively down-regulated, which might be associated with alterations in MHC IIa/IIx content. It is interesting that only MyoD is responsive, suggesting that this protein might be a key player in diaphragm function in HF. These changes are likely to contribute to diaphragm alterations caused by HF. Further studies are needed to map the complexity of this phenomenon and develop strategies to minimize the effects of HF on diaphragm myopathy.

Acknowledgements

The authors thank José Carlos Georgette and Sueli Cruz Michelin for technical assistance. This work is part of the MSc thesis presented by FLP to Universidade Estadual de Campinas – UNICAMP, in 2005. Grant support: FAPESP (Proc: 04/01516-1).

References

Acharyya S., Ladner K.J., Nelsen L.L. *et al.* (2004) Cancer cachexia is regulated by selective targeting of skeletal muscle gene products. *J. Clin. Invest.* **114**, 370–378.

Allen D.L., Sartorius C.A., Sycuro L.K., Leinwand L.A. (2001) Different pathways regulate expression of the skeletal myosin heavy chain genes. *J. Biol. Chem.* **276**, 43524–43533.

Alway S.E., Degens H., Lowe D.A., Krishnamurthy G. (2002) Increased myogenic repressor Id mRNA and protein levels in hindlimb muscles of aged rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **282**, R411–R422.

Anker S.D., Ponikowski P.P., Clark A.L. *et al.* (1999) Cytokines and neurohormones relating to body composition alterations in the wasting syndrome of chronic heart failure. *Eur. Heart J.* **20**, 683–693.

Bernocchi P., Cargnoni A., Vescovo G. *et al.* (2003) Skeletal muscle abnormalities in rats with experimentally induced heart hypertrophy and failure. *Basic Res. Cardiol.* **98**, 114–123.

Carlsen H. & Gundersen K. (2000) Helix-loop-helix transcription factors in electrically active and inactive skeletal muscle. *Muscle Nerve* **23**, 1374–1380.

Carvalho R.F., Cicogna A.C., Campos G.E. *et al.* (2003) Myosin heavy chain expression and atrophy in rat skeletal muscle during transition from cardiac hypertrophy to heart failure. *Int. J. Exp. Pathol.* **84**, 201–206.

Carvalho R.F., Cicogna A.C., Campos G.E. *et al.* (2006) Heart failure alters MyoD and MRF4 expressions in rat skeletal muscle. *Int. J. Exp. Pathol.* **87**, 219–225.

Dalla Libera L., Zennaro R., Sandri M., Ambrosio G.B., Vescovo G. (1999) Apoptosis and atrophy in rat slow skeletal muscle in chronic heart failure. *Am. J. Physiol., Cell Physiol.* **277**, C982–C986.

Dalla Libera L., Ravara B., Angelini A. *et al.* (2001) Beneficial effects on skeletal muscle of the angiotensin II type 1 receptor blocker irbesartan in experimental heart failure. *Circulation* **103**, 2195–2200.

Dalla Libera L., Ravara B., Volterrani M. *et al.* (2004) Beneficial effects of GH/IGF-1 on skeletal muscle atrophy and function in experimental heart failure. *Am. J. Physiol., Cell. Physiol.* **286**, C138–C144.

De Sousa E., Veksler V., Bigard X., Mateo P., Serrurier B., Ventura-Clapier R. (2001) Dual influence of disease and increased load on diaphragm muscle in heart failure. *J. Mol. Cell. Cardiol.* **33**, 699–710.

Eftimie R., Brenner H.R., Buonanno A. (1991) Myogenin and MyoD join a family of skeletal muscle genes regulated by electrical activity. *Proc. Natl Acad. Sci. USA* **88**, 1349–1353.

Ekmark M, Gronevick E, Schjerling P, Gundersen K. (2003) Myogenin induces higher oxidative capacity in pre-existing mouse muscle fibers after somatic DNA transfer. *J. Physiol.* **548**, 259–269.

Guttridge D.C., Mayo M.W., Madrid L.V., Wang C.Y., Baldwin A.S. Jr (2000) NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. *Science* **289**, 2363–2366.

Hinterberger T.J., Sassoon D.A., Rhodes S.J., Konieczny S.F. (1991) Expression of the muscle regulatory factor MRF4 during somite and skeletal myofiber development. *Dev. Biol.* **147**, 144–156.

Hughes S.M., Taylor J.M., Tapscott S.J., Gurley C.M., Carter W.J., Peterson C.A. (1993) Selective accumulation of MyoD and myogenin mRNAs in fast and slow muscle is controlled by innervation and hormones. *Development* **118**, 1137–1147.

Hughes S.M., Koyshi K., Rudnicki M., Maggs A.M. (1997) MyoD protein is differentially accumulated in fast and slow skeletal muscle fibres and required for normal fibre type balance in rodents. *Mech. Dev.* **61**, 151–163.

Hughes S.M., Chi M.M., Lowry O.H., Gundersen K. (1999) Myogenin induces a shift of enzyme activity from glycolytic to oxidative metabolism in muscles of transgenic mice. *J. Cell Biol.* **145**, 633–642.

Israël A. (2000) The IKK complex: an integrator of all signals that activate NF-kappaB? *Trends Cell Biol.* **10**, 129–133.

Leineweber K., Brandt K., Wludyka B. *et al.* (2002) Ventricular hypertrophy plus neurohumoral activation is necessary to alter the cardiac beta-adrenoceptor system in experimental heart failure. *Circ. Res.* **91**, 1056–1062.

Levine B., Kalman J., Mayer L., Fillit H.M., Packer M. (1990) Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N. Engl. J. Med.* **323**, 236–241.

Li X., Moody M.R., Engel D. *et al.* (2000) Cardiac-specific overexpression of tumor necrosis factor-alpha causes

oxidative stress and contractile dysfunction in mouse diaphragm. *Circulation* **102**, 1690–1696.

McMurray J., Abdullah I., Dargie H.J., Shapiro D. (1991) Increased concentrations of tumor necrosis factor in 'cachectic' patients with severe chronic heart failure. *Br. Heart J.* **66**, 356–358.

Mozdziak P.E., Greaser M.L., Schultz E. (1998) Myogenin, MyoD, and myosin expression after pharmacologically and surgically induced hypertrophy. *J. Appl. Physiol.* **84**, 1359–1364.

Murre C., McCaw P.S., Vaessin H. *et al.* (1989) Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. *Cell* **58**, 537–544.

Parker M.H., Seale P., Rudnicki M.A. (2003) Looking back to the embryo: defining transcriptional networks in adult myogenesis. *Nat. Rev. Genet.* **4**, 497–507.

Racz G.Z., Gayan-Ramirez G., Testelmans D. *et al.* (2003) Early changes in rat diaphragm biology with mechanical ventilation. *Am. J. Respir. Crit. Care Med.* **168**, 297–304.

Reindel J.F., Ganey P.E., Wagner J.G., Slocombe R.F., Roth R.A. (1990) Development of morphologic, hemodynamic, and biochemical changes in lungs of rats given monocrotaline pyrrole. *Toxicol. Appl. Pharmacol.* **106**, 179–200.

Seward D.J., Haney J.C., Rudnicki M.A., Swoap S.J. (2001) bHLH transcription factor MyoD affects myosin heavy chain expression pattern in a muscle-specific fashion. *Am. J. Physiol., Cell Physiol.* **280**, C408–C413.

Simonini A., Massie B.M., Long C.S., Qi M., Samarel A.M. (1996) Alterations in skeletal muscle gene expression in the rat with chronic congestive heart failure. *J. Mol. Cell. Cardiol.* **28**, 1683–1691.

Siu P.M., Donley D.A., Bryner R.W., Alway S.E. (2004) Myogenin and oxidative enzyme gene expression levels are elevated in rat soleus muscles after endurance training. *J. Appl. Physiol.* **97**, 277–285.

Smith C.K. II, Janney M.J., Allen R.E. (1994) Temporal expression of myogenic regulatory genes during activation, proliferation, and differentiation of rat skeletal muscle satellite cells. *J. Cell. Physiol.* **159**, 379–385.

Staib J.L., Swoap S.J., Powers S.K. (2002) Diaphragm contractile dysfunction in MyoD gene-inactivated mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **283**, R583–R590.

Tikunov B.A., Mancini D., Levine S. (1996) Changes in myofibrillar protein composition of human diaphragm elicited by congestive heart failure. *J. Mol. Cell. Cardiol.* **28**, 2537–2541.

Tikunov B.A., Mancini D., Levine S. (1997) Chronic congestive heart failure elicits adaptations of endurance exercise in diaphragmatic muscle. *Circulation* **95**, 910–916.

Vescovo G., Jones S.M., Harding S.E., Poole-Wilson P.A. (1989) Isoproterenol sensitivity of isolated myocytes from rats with monocrotaline-induced right-sided hypertrophy and heart failure. *J. Mol. Cell. Cardiol.* **21**, 1047–1061.

Vescovo G., Ceconi C., Bernocchi P. *et al.* (1998) Skeletal muscle myosin heavy chain expression in rats with monocrotaline-induced cardiac hypertrophy and failure. Relation to blood flow and degree of muscle atrophy. *Cardiovasc. Res.* **39**, 233–241.

Voytik S.L., Przyborski M., Badylak S.F., Konieczny S.F. (1993) Differential expression of muscle regulatory factor genes in normal and denervated adult rat hindlimb muscle. *Dev. Dyn.* **198**, 214–224.

Wheeler M.T., Snyder E.C., Patterson M.N., Swoap S.J. (1999) An E-box within the MHC IIB gene is bound by MyoD and is required for gene expression in fast muscle. *Am. J. Physiol.* **276**, C1069–C1078.

Wilson J.R. & Mancini D.M. (1993) Factors contributing to the exercise limitation of heart failure. *J. Am. Coll. Cardiol.* **22**, 93A–98A.