Morphological Analysis of Skeletal Muscle in Patients with Chronic Heart Failure

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Abstract

The present study was designed to define the qualitative and quantitative morphological changes of skeletal muscle in patients with chronic hearth failure (CHF). Needle biopsies were obtained from the right vastus lateralis muscle in 14 patients (mean age 51 years) with stable CHF (NYHA class III) and in 6 healthy controls. In CHF patients the mean maximal oxygen consumption (VO_2 max) was reduced as well as the isometric maximum voluntary muscle contraction (MCV). All studied biopsies were abnormal; there was a generalized reduction in the fibre diameter with predominant type 2 fibre atrophy. The fibre type distribution was shifted to type 2 fibres. An increase in intracellular lipids was seen by histochemical and ultrastructural methods. In two cases, findings included increased acid phosphatase activity, a marker of cell degeneration and necrosis. In CHF patients, a significant reduction in the mitochondrial percentage per fibre area was also observed. The capillary density of muscle increased in the cases in which numerous fibres were atrophic, but the capillary/ fibre ratio was normal. While some findings appear as changes resulting from prolonged deconditioning, immobilisation or inactivity of patients, the complex of the morphological alterations seems to indicate intrinsic skeletal muscle alterations emerging in CHF. The significant reduction in mitochondrial percentage and the increase in lipids indicating low utilisation of lipid energy sources, may contribute to muscle fibre compensatory adaptation with increase of glycolitic anaerobic metabolism.

Key words: chronic heart failure, blood flow, skeletal muscle, muscle fibre types, mitochondria, capillary supply, morphology.

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Morphological changes of skeletal muscle have been previously described in patients with cardiomyopathies. These findings indicate that a lot of patients with dilated and hypertrophic cardiomyopathy had electrophysiological and morphological patterns of myopathy with unknown nature suggestive of coexistent cardiac and skeletal muscle pathology [1, 3]. Abnormalities of both cardiac and skeletal muscle were also described in patients with cardiac dysrhythmias, ventricular tachycardia and congestive heart failure [7, 8].

Recent investigations using ultrastructural and cytochemical methods, and ^{31}P nuclear magnetic resonance (NMR) spectroscopy have demonstrated skeletal muscle alterations in patients with CHF and its relation to exercise intolerance and abnormal muscle metabolism has been proposed [5, 14, 15, 22]. Moreover, studies based on metabolic and anthropometric evaluations indicated skeletal muscle atrophy in about 70 per cent of patients with stable CHF (NYHA class II and III) [2].

In order to contribute to a better definition of skeletal muscle changes, we have studied 14 patients with stable chronic heart failure (NYHA Class III). The assessment of skeletal muscle function was based on qualitative and quantitative analysis of muscle fibres, on enzyme-histochemical and ultrastructural parameters in needle *vastus lateralis* muscle biopsies.

Patients and Methods

Fourteen patients (seven male), mean age 51.6 years (range 28-70), with stable chronic heart failure (New York Heart Association functional class III) were selected for this study. The cause of heart failure was ischaemic coronary artery disease (HID) in 8 patients; idiopathic dilated cardiomyopathy (IDC) in 3 patients and aortic or mitral alterations (VD) in 3 patients. The selection of patients was

made on the basis of the following criteria: a) negative family and personal history for neuromuscular diseases; b) clinical examination excluding varicose veins and excess of adipose tissue in the legs; c) signs of exercise intolerance due to fatigue or breathlessness; d) clinical findings indicating stable chronic heart failure with more than 4 months duration. Exercise testing of maximal oxygen consumption (VO_2 max) using the Naughton protocol [11] and the evaluation of the muscle strenght as maximal voluntary muscle isometric contraction (MCV max) using the Edwards muscle testing chair method [9] indicate in all patients severe limitation of exercise capacities.

A needle biopsy was obtained from the lateral portion of the right *vastus lateralis* muscle under local anaesthesia in CHF patients and in 6 healthy control subjects. Some muscle specimens were frozen in isopentane cooled in liquid nitrogen and transverse cryostat sections (8 μm) were stained with hematoxylin and eosin, and Gomori trichrome stain, with histochemistry (Oil red O, PAS stain) and with enzyme-histochemical reactions (myosin- ATPase at pH 9.6 and 4.6, COX, NADH and acid phosphatase). Quantitative analysis of muscle fibres was made on sections stained for myosin ATPase using an automatic computerized image analyzer (IBAS I-II, Kontron, Munich, FGR). The percentage and mean diameter of type I and 2 fibres [6] were considered. Other specimens were fixed in 12% neutral formalin and paraffin embedded transverse sections were stained with Gomori silver impregnation for reticulin and the muscle capillaries were identified by counting using a previously described method [21]. The numerical density of capillaries (CD) was evaluated with a frame in the eyepiece of the microscope at 40X objective. The capillaries fibre ratio (C/F) was calculated as capillary number within the frame/number of fibres within the frame. Small muscle specimens were fixed in a glutaraldehyde-paraformaldehyde mixture in 0.1 sodium cacodylate buffer (Karnovsky fluid) and then treated for electron microscopy. Quantitative analysis on mitochondria (size and % per fibre area) and on intracellular lipid droplets (% per fibre area) was performed on five micrographs at 12,000X. The micrographs were taken from ultrathin longitudinal sections of the central part of the muscle fibre. Statistical analysis of data (count number; number of size classes; minimal, maximal and mean value; standard deviation) was performed by means of the IBAS I system.

Results

Table 1 report the measures of VO_2 max consumption and of MCV max of the vastus lateralis muscle in CHF patients. All patients showed reduced exercise capacity (VO_2 max: mean 11.9; range 10.2-13.6 ml.Kg-1; normal value: 28) and a reduced muscle strength (53.7%; range 38-70%).

In 11 CHS patients, the morphological analysis of muscle biopsies revealed abnormal variation in the fibre size; two biopsies showed fibre necrosis indicated by increased acid phosphatase activity (Fig 1) and twelve increased intracel-

Table 1. Skeletal muscle strength measured as maximal oxygen consumption (VO_2 max) and maximal voluntary contraction (MCV) of vastus lateralis muscle in patients with chronic heart failure.

Patient	Age	Sex	Diagnosis	VO_2 max (ml.Kg-1.min-1)	MCV (% normal)
1	60	F	HID	13.6	64
2	50	F	HID	13.4	66
3	42	M	VD	13.0	66
4	50	F	VD	10.6	44
5	61	F	VD	12.6	48
6	61	F	HID	10.8	38
7	57	M	HID	12.6	66
8	58	M	IDC	13.0	60
9	28	M	IDC	10.0	46
10	56	F	HID	11.6	58
11	50	M	IDC	13.4	48
12	64	F	HID	11.6	52
13	70	M	HID	10.2	42
14	68	M	HID	10.6	44
Mean values				11.9	53.7
Normal values				28	100

HID = ischaemic heart disease; IDC = idiopathic dilated cardiomyopathy; VD = valvular disease

lular lipid storage after Oil red O histochemical stain. In all cases no abnormalities in COX and DPNH-diaphorase distribution were observed.

In Table 2 are reported for each patient the mean fibre type diameter, the percentage of type 1 fibres, the mean of capillary density (CD) and the capillary/fibre ratio (C/F). Normal values derived from 6 healthy subjects were also reported. The enzyme-histochemical characterization of fibre types indicated significant atrophy of both type 1 and 2 fibres, with patterns of predominant type 2 fibre atrophy (fig 2); in most cases the fibre type distribution was shifted to type 2 fibres. The C/F was within the normal range and the capillary density was higher than normal in the cases in which the muscle atrophy was marked. The number and size of mitochondria and the number of intracellular lipid droplets are reported in Table 3. The mitochondrial size and distribution in muscle fibres were normal but a significant reduction in mitochondrial percentage per fibre area was observed. The mean number of lipid droplets per fibre area was significantly increased if compared with normal muscle fibres; the distribution of lipid droplets in the intermyofibrillar and subsarcolemmal areas was generally uniform .

Discussion

It has been demonstrated that exertional fatigue and limitation of exercise capacity are important limiting symptoms in patients with CHF. These limitations correlates poorly with hemodynamic abnormalities suggesting that additional changes in skeletal muscle play a pathophysiological role in the exercise intolerance [16]. Moreover recent MRS and MRI studies indicate that patients with CHF developed significant skeletal muscle atrophy, that contributes modestly to the exercise intolerance and to muscle metabolic abnormalities seen in such subjects [15]. Previous studies on skeletal muscle biopsies from patients with severe congestive CHF revealed morphological abnormalities as increased acid phosphatase activity, excess of intracellular lipids, atrophy of both type 1 and 2 fibres with marked variation in fibre size. Muscle capillary density and the activity of some mitochondrial enzymes were normal [12], but recent biochemical investigations indicate in CHF patients a significant reduction of cytochrome oxidase activity in mitochondria and ultrastructural studies showed reduction of the volume density of mitochondria and of the surface density of mitochondrial cristae [5].

Table 2. Biopsy findings and morphometry in vastus lateralis muscle from patients with CHF (Mean ± SD).

Patient N°	Fibre diameter (μ) Type 1	Fibre diameter (μ) Type 2	Type 1 fibre %	Capillaries per fibre (C/F)	Capillaries per square mm of m.fibre	
1	50 ± 4.2	36 ± 2.4	24	1.38 ± 0.08	340 ± 28	
2	46 ± 2.6	30 ± 2.0	26	1.32 ± 0.04	360 ± 33	
3	54 ± 3.2	52 ± 2.6	46	1.40 ± 0.06	260 ± 26	
4	44 ± 2.6	42 ± 3.2	39	1.30 ± 0.08	280 ± 22	
5	42 ± 2.0	30 ± 1.6	42	1.38 ± 0.06	360 ± 42	
6	48 ± 3.2	32 ± 2.0	38	1.28 ± 0.10	350 ± 26	
7	48 ± 2.8	34 ± 2.4	40	1.36 ± 0.12	330 ± 34	
8	58 ± 2.6	56 ± 2.6	46	1.30 ± 0.08	270 ± 28	
9	52 ± 3.0	52 ± 2.4	50	1.40 ± 0.04	300 ± 22	
10	42 ± 1.6	42 ± 2.0	46	1.36 ± 0.08	280 ± 18	
11	48 ± 2.0	30 ± 1.8	39	1.30 ± 0.10	300 ± 16	
12	48 ± 2.2	30 ± 1.4	44	1.26 ± 0.06	360 ± 28	
13	42 ± 2.8	34 ± 2.0	36	1.32 ± 0.06	290 ± 36	
14	48 ± 2.4	32 ± 2.2	30	1.20 ± 0.04	330 ± 31	
CHF	47.8 ± 4.2	34 ± 9.2	39.5	1.32 ± 0.05	315 ± 36	Mean ± SD
	(42-58)	(30-56)	(24-50)	-	-	Min-max
	16	26	26			Range
Normal	55.5 ± 4.6	52 ± 5.4	47	1.39 ± 0.02	277 ± 18	Mean ± SD
	(50-62)	(45-58)	(40-51)	-	-	Min-max
	26	13	11			Range
	P = 0.003	P = 0.003	P = 0.060	P = 0.010	P = 0.027	

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A New Electrophoretic Micromethod for Assessing Myosin Heavy Chain Composition of Skeletal Muscle in Chronic Heart Failure

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Abstract

Chronic heart failure is a clinical disorder characterized by exercise intolerance. Fatigue and shortness of breath are the symptoms causing a patient to stop exercising. It is clear from previous studies that in this syndrome the skeletal muscle of the lower limbs develops a myopathy with atrophy and shift from the slow type to the fast type fibres.

We devised a new electrophoretic micromethod that is able to separate, from 50-150 µg needle biopsies of the gastrocnemius muscle, the three different isoforms of Myosin Heavy Chains (MHC). A 7.5% polyacrylamide SDS PAGE with 37.5% glycerol was used. About 1 µg of protein was loaded on each well and silver stain was used to detect the isolated isoforms. The percentage of MHC1 (slow isoform), MHC2A (fast oxidative) and MHC2B (fast glycolytic) was determined by densitometric scan. We studied 19 patients with chronic heart failure (age 71.2 ± 7.6) whose ejection fraction was 42.5 ± 15.2. MHC1 was 51.83 ± 15.04 whilst MHC2B was 23.5 ± 7.4 and MHC2A 24.83 ± 15.01. Within the CHF group there was a positive correlation between NYHA class and MHC2A ($r = 0.47$, $p < 0.05$) and MHC2B ($r = 0.55$, $p < 0.01$) and a negative correlation between NYHA class and MHC1 ($r = 0.74$, $p < 0.001$). Similarly, significant correlations were found for ejection fraction, diuretic consumption score, exercise test tolerance and degree of muscle atrophy.

These data seem to suggest the hypothesis that the myopathy present in patients with chronic heart failure is specific and that the severity of the disease correlate with the magnitude of the MCH redistribution.

The micromethod used in this study to evaluate MHC isoforms seems to be very reproducible and well tolerated by the patients so that it allows to take several biopsies and therefore enabling thorough follow up.

Key words: myosin heavy chain, heart failure, skeletal muscle, electrophoresis.

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Chronic Heart Failure (CHF) is a clinical disorder characterized by exercise intolerance. Fatigue or shortness of breath are the symptoms causing a patient to stop exercising. It has been shown that lower limbs skeletal muscle performance is disordered in CHF and this can at least in part explain symptoms complained by the patient [21]. Lipkin and Poole-Wilson [17] initially suggested that *quadriceps femoris* muscle strength is reduced because of loss of skeletal muscle bulk, although the mean force per unit area was within the normal range [21, 22].

Muscle wasting in CHF is accompanied by profound histologic changes. Lipkin et al. [17] reported results of the quadriceps muscle biopsies showing a shift towards type II fibres with presence of atrophic fibres. Mancini et al. [20] studied the *gastrocnemius* reporting a shift toward type IIB fibres and atrophy of the type IIA. Sullivan and Cobb [25] examined needle biopsies from *vastus lateralis* and found type I fibres reduced and IIB to be increased. Drexler et al. [10] looked into the possible contribution of mitochondrial abnormalities to the myopathy of CHF. In

the *vastus lateralis* they found a reduction in volume density of mitochondria and surface density of christae. They also found a reduction of type I and a relative increase of type II fibres.

We tried to correlate the severity of the disease with the magnitude of the shift in MHC pattern. We did that by means of a new electrophoretic micromethod devised in our laboratories and able to separate the three skeletal muscle MHC isoforms, namely the slow MHC1 isomyosin, the MHC2A (fast oxidative) and the MHC2B (fast glycolytic).

Patients and Methods

Patients

Nineteen patients with CHF ((10 males, 9 females) (9 with Dilated Cardiomyopathy (DCM), 4 with Hypertensive Heart Disease (HHD) and 6 with Ischaemic Heart Disease (IHD)) were studied. These were all patients with previous established diagnosis done by means of clinical history, ECG, exercise test, echo and coronary angiogram. Nine control patients were also studied.

Skeletal muscle biopsy

Skeletal muscle biopsies were taken from the *gastrocnemius* muscle by using a 17G Histo-Cut soft tissue disposable Menghini needle (Sterylab Inc. Milan, Italy). From each specimen between 50 and 150 μg of tissue were obtained. The specimen was immediately frozen in liquid nitrogen and kept in a -80°C fridge until processed. No anaesthesia was employed.

Exercise test tolerance

All the patients with CHF and the normal subjects underwent a treadmill exercise test according to a modified Naughton protocol. The protocol envisaged seven three minutes each stages at a fixed speed of 3 Km/h starting from 0% grade (stage 0) with increments of 3.5%. All subjects exercised to a symptom limited maximum or until they reached theoretical submaximal heart rate.

Echocardiographic measurements

All the CHF and control subjects had a 2D-Echo and color-Doppler. Ejection Fraction (EF), Left Ventricle End Diastolic Volume (LVEDV), Left Ventricle End Systolic Volume (LVESV), A wave/E wave ratio were calculated with a parasternal short axis approach. EF was calculated with the modified Simpson Method.

NYHA class

NYHA class was assessed in all the patients.

Diuretic consumption

Patients, according to Harding et al. [14] were divided into 5 classes according to diuretic consumption. Class 1 was no diuretic, class 2 20 to 40 mg of frusemide or a thiazide diuretic a day, class 3 ≥ 40 mg frusemide, class 4 ≥ 80 mg, class 5 ≥ 120 mg.

Electrophoretic separation of MHC

The method described by Carraro et al. was used [3, 23]. The muscle biopsies were solubilized in a small volume (100-200 μl) of 2.3% SDS, 10% glycerol, 0.5% 2-mercaptoethanol, 62.5 mM TRIS-HCl pH = 6.8 and boiled for 5 minutes. The running buffer was added to the sample in a ratio of 1:1 v/v. Analytical SDS page of MHC was performed on 7% polyacrylamide slabs, 0.75 x 130 x 130 mm. Slabs were prepared according to Laemmli [15], but 37.5% v/v glycerol was present both in the separating and in the stacking gel. The stacking gel was composed of 37.5% glycerol, 4% T acrylamyde-Bis (36.5:1), 12.5 mM TRIS-HCl (pH = 6.8), 0.1% SDS. The separating gel was composed of 37.5% glycerol, 7% T acrylamyde-Bis (36.5:1), 375 mM TRIS-HCl (pH = 8.8), 0.1% SDS. Running buffer was made of 50 mM TRIS, 384 mM glycine, pH = 8.3, 0.2% SDS (without correction of pH with HCl). Separation of MHC was achieved using constant current at 4 mA (corresponding to about 40 V). After 6-8 hours the running buffer was changed and electrophoresis restarted with the same parameters. After 24 hours the voltage rises to about 130-160 V and the run is stopped. Gels containing approximatively 0.2 μg of protein per band are stained with 0.1% Coomassie brilliant blu in 5% acetic acid, 40% methanol and destained with 40% methanol, 7% acetic acid. Gels with less than 0.1 μg protein were stained with Silver method. In a previous series of experiments [23] MHCs after SDS-page were immunoblotted with anti MHC1-2A mAb (BF32) and anti- MHC2X-2B (D9) demonstrating the good reproducibility of MHC separation.

Assessment of MHC content

The percent distribution of MHC1, MHC2A and MHC2B was determined by densitometric scan of the slab gel after staining with Coomassie brilliant blue. The scan was performed by using a GS300 Transmittance Reflectance Scanning Densitometer (Hoefer Scientific Instruments) connected to a McIntosh SE Apple Computer. The data were processed by using the GS370 Densitometry Software.

Statistical analysis

Coefficient of variation, and linear regression were used when appropriate.

Results

Age

Age was 71.2 ± 7.6 in the CHF group and 55.4 ± 8.5 in the controls (p = NS).

NYHA class

Between CHF patients 4 were in NYHA class I, 4 in NYHA II, 8 in NYHA III and 3 in NYHA IV.

Haemodynamic parameters

EF was 42.5% ± 15.2 (n = 19) in CHF patients and 60.3 ± 1.4 in controls (p < 0.001).

Diuretic consumption

Between CHF patients diuretic consumption score was 1 in 9 subject, 2 in 7, 3 in 6, 4 in 4 and 5 in 2.

Myosin Heavy Chain composition (MHC)

Myosin Heavy Chain composition (MHC) was 51.83 ± 15.04 for MHC1, 24.83 ± 15.01 for MHC2A and 23.5 ± 7.45 for MHC2B in the CHF group.

Correlation between MCH composition and parameters of clinical severity of CHF

Clinical parameters indicating the severity of heart failure were correlated with MHC composition in patients with CHF and controls.

a) NYHA class

There was a significant, negative correlation between percentage of MHC1 and NYHA class ($r = 0.74$, $p < 0.001$), whilst there was a positive correlation between NYHA class and MHC2A ($r = 0.47$, $p < 0.05$) and MHC2B ($r = 0.55$, $p < 0.01$) (Fig. 1).

b) Ejection Fraction (EF)

EF correlated conversely in significant, positive manner with MHC1 ($r = 0.65$, $p < 0.001$) and in a negative fashion with MHC2A ($r = 0.75$, $p < 0.001$). Although there was a trend for MHC2B to decrease in patients with higher EF, this did not reach statistical significance ($r = 0.26$, $p = NS$) (Fig. 2).

c) Diuretic consumption

Diuretic consumption score correlated significantly in a negative manner with MHC1 ($r = 0.62$, $p < 0.001$) and in a positive one with MHC2A ($r = 0.35$, $p < 0.1$) and MHC 2B ($r = 0.49$, $p < 0.001$) (Fig. 3).

d) Exercise Test Tolerance (ETT)

There was a significant correlation between steps reached on the treadmill and MHC composition. The correlation was in fact positive with MHC1 ($r = 0.65$, $p < 0.001$) and negative with MHC2A ($r = 0.37$, $p < 0.05$) and MHC 2B ($r = 0.57$, $p < 0.01$) (Fig. 4).

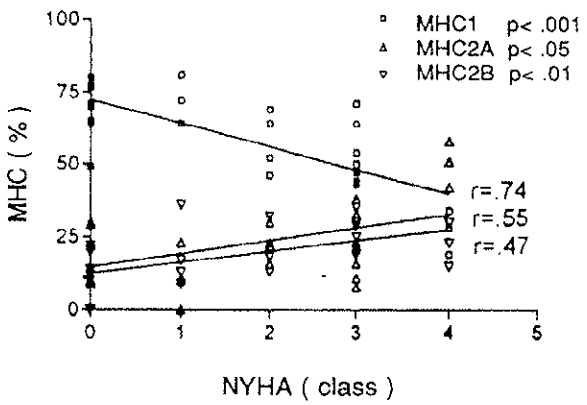

Figure 1. Correlation between Myosin Heavy Chain (MHC) composition and NYHA class.

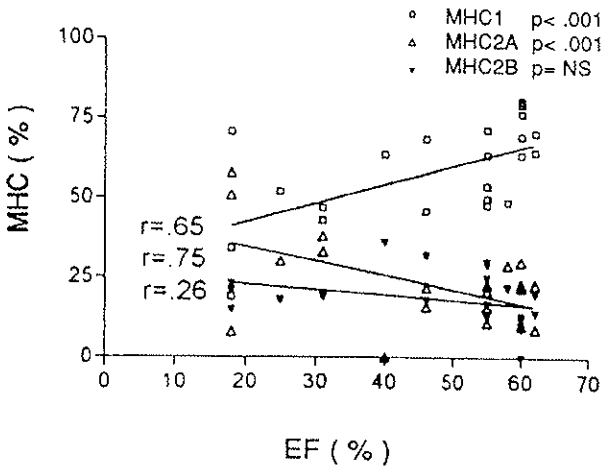

Figure 2. Correlation between Myosin Heavy Chain (MHC) composition and Ejection Fraction (EF).

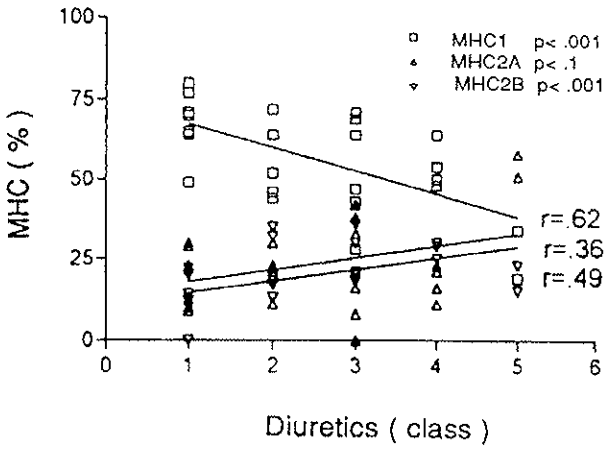

Figure 3. Correlation between Myosin Heavy Chain (MHC) composition and Diuretic Consumption.

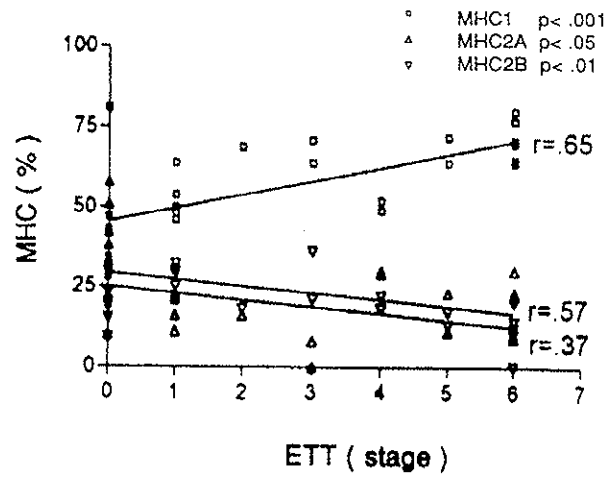

Figure 4. Correlation between Myosin Heavy Chain (MHC) composition and Exercise Test Tolerance (ETT).

Reproducibility of the method

Five biopsies were taken from the same subject with CHF and processed separately. On the same slab gel samples from the 5 different biopsies were loaded. The coefficient of variation between the 5 samples was 5% for MHC1, 5% for MHC2A and 7% for MHC2B.

We also loaded on 5 separate SDS-pages samples coming from one patient. Electrophoresis were carried out on separate days. The sample, once solubilized in SDS, 2-mercaptoethanol and TRIS-HCl, was kept in a fridge at -10°C until the experiment was done. The coefficient of variation was 4% for MHC1, 6% for MHC2A and 4% for MHC2B.

Discussion

The novelty of this study is the method we used for separating myosin heavy chains. It allows separation of the three isoforms from extremely small tissue samples. The reproducibility of the method is fairly high, in fact the coefficient of variation of MHC percent distribution from different biopsies from the same patient is low, as well as that found for the repeated Electrophoresis of the same bioptic sample.

We found a correlation between indicators of severity of CHF and MHC composition. We observed in fact a significant correlation between NYHA class, exercise tolerance, diuretic consumption and ejection fraction with the percentage of MHC isoforms. It looks as if patients with CHF have a specific myopathy the severity of which is related to that of the heart failure syndrome. Our data show that in patients with cardiac failure there is a shift toward the fast isoforms MHC2A and MHC2B, with a significant decrease of the slow isoform MHC1. In fact in a study where CHF patients were compared to normal subjects we found significant differences between these two groups of patients [28]. Our data are in agreement with those of Poole-Wilson [17], Mancini [20] and Sullivan [25] who described an increased percentage of easily fatigable, fast twitch IIB fibres and atrophy of type I slow twitch fibres. Similar results have been reported by Sabbah et al. [24] in dogs with chronic heart failure. These changes have been ascribed to intrinsic skeletal muscle abnormalities [8] and only in part to muscle underperfusion [21]. Moreover Wilson et al. [29] suggested that exertional fatigue is due to skeletal muscle abnormalities and not to reduced blood flow. Atrophy due to muscle deconditioning (disuse hypothesis) [9, 12, 27] has been claimed to be partially responsible for these muscle alterations. In term of the existence of a specific skeletal muscle myopathy with selective type I fibres atrophy, this has been reported by Caforio et al. [2] in patients with cardiomyopathies. These changes resamble alterations found in congenital and idiopathic myopathies but were found by these Authors to be unrelated to NYHA class. Conversely Dunnigan et al. [11] in patients with cardiomyopathy described a type II skeletal muscle fibre atrophy. Changes in skeletal muscle in CHF are very much the same of those observed in the muscle denervation in the rat [5]. In this experimental model in fact denervation is accompanied by an increased expression of adult fast myosins [5]. The synthesis of adult slow myosins is dependent on the actual activity of motor innervation, while adult fast is expressed in denervated fibres even though the neuronal influences are removed [1, 4]. This hypothesis is supported by observations in paraplegic patients where a slow to fast transformation occurs [13, 18]. Other Authors suggest different mechanisms. For instance, circulating factors such as increased concentrations of Tumor Necrosis Factor [19] or metabolic and vascular alterations like insulin resistance [26] or increased thickness of the endothelial cells [16] can be invoked as determinants of muscle changes. However a "neural theory" has been recently put forward to explain symptoms and muscle waste in CHF. Coats et al. [7] have in fact proposed the "ergo- and metabolo- receptors" theory, which envisages the existence of chemically sensitive receptors able to pick up muscle work and metabolism. They transmit to the central nervous system via small unmyelinated nerve fibres stimuli that can drive blood pressure responses and increase sympathetic activity. An increased ergoreceptor activity in patients with CHF might maintain the increased sympathetic outflow with consequent increased peripheral resistances, decreased perfusion and further muscle deterioration.

Fine needle biopsy is extremely easy to be performed. It does not cause any harm to the patient and is so well tolerated that does not require any sort of anaesthesia. We did not have any sort of complication in our series of patients. The technique allows serial bioptic sampling in the same patients enabling us to study either muscle worsening during the progression of the disease or possible improvements due to different therapeutic approaches such as training or hormonal therapy that seem able to restore muscle bulk, relief symptoms and increase exercise tolerance [6]. The study of MHC composition may also have the potential to detect early changes in skeletal muscle composition and correlate them with humoral, biochemical and functional parameters.

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