

Skeletal Muscle Adaptation in Patients with Severe Heart Failure: Implications for Aorto- and Cardiomyoplasty

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Abstract

Cardiomyoplasty is a surgical technique to improve cardiac pump function in patients with severe heart failure, using the Latissimus Dorsi (LD) muscle. However, intrinsic skeletal muscle alterations have been reported in patients suffering from chronic heart failure.

Therefore, the aim of the present study was to compare histological characteristics of the LD muscle in patients with chronic heart failure (HF), with mild heart failure patients and non heart failure individuals. The first group (chronic-HF) were patients submitted for a Cardiomyoplasty procedure ($n = 6$, NYHA class IV, age 57 ± 5). The second group (mild-HF) were patients with mild heart failure ($n = 7$, NYHA class II, age 73 ± 8) and the third group (non-HF) were patients without signs of heart disease and therefore considered as a reference group ($n = 10$, age 74 ± 9). The LD muscle was examined for fibre type distribution, fat content, connective tissue content, and the capillary to fibre ratio.

The latissimus dorsi muscle revealed a significant decrease of type I muscle fibres in chronic HF as compared to non-HF ($32\% \pm 11$ vs $52\% \pm 14$). Percentage type I fibres in mild-HF was $46\% \pm 13$, demonstrating the tendency of reduced percentage type I fibres due to mild heart failure. In the latissimus dorsi no differences in fat content, connective tissue content or capillary to fibre ratio was observed between the three groups.

In conclusion, these findings indicate that the fibre type distribution of the LD muscle in patients with chronic heart failure, is significantly altered. This reduced amount of type I fibres in the latissimus dorsi muscle is the opposite of what is required for optimal cardiac assist in aorto- and Cardiomyoplasty. Therefore, it should be subject of future research whether this may retard transformation in a fatigue resistant muscle.

Key words: heart failure, musculus latissimus dorsi, histology, fibre type, human.

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Exercise intolerance and early fatigue are the primary symptoms in patients with mild or severe congestive heart failure. In order to explain the low level of exercise tolerance in these patients, several investigators demonstrated that patients with chronic heart failure exhibit intrinsic skeletal muscle changes, including a fibre type redistribution and a decrease in the number of capillaries per fibre [3, 8, 13, 20, 22]. These changes can explain the early onset of the anaerobic threshold in patients with chronic heart failure.

Alterations in skeletal muscle morphology due to heart failure are an important item for aorto- and Cardiomyoplasty, where the latissimus dorsi (LD) muscle is used for cardiac assist after conditioning [9, 11, 14, 16]. Pre-operative vitality of the LD muscle may be an important determinant of the efficacy of the procedure.

Therefore, the present study was designed to assess the effect of chronic heart failure on the LD muscle. This was done by comparing two groups of patients with increasing heart failure with normal subjects. Histological analyses were used to assess the vitality of the latissimus dorsi muscle, by comparing two groups of patients with increasing heart failure with normal subjects. Histological analyses were used to assess the vitality of the latissimus dorsi muscle.

Materials and Methods

Study subjects

The study population consisted of 3 groups of patients. Tissue specimen were obtained from the middle of the lateral aspect of the left LD muscle in all patients. The first

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group consisted of 6 patients suffering from chronic heart failure (Chronic-HF, NYHA class IV), who were submitted for a cardiomyoplasty procedure, and the biopsy was taken peroperatively. Patients in the other two groups underwent autopsy at the Department of Pathology. Biopsy specimens of the LD muscle were obtained within 24 hours after death occurred. Based on both clinical information and echocardiography data these patients were divided into two groups: mild-HF or non-HF. Those with signs of a modestly reduced ejection fraction (EF), shown by echocardiography or other relevant clinical information, were enrolled in the mild heart failure group (mild-HF). Patients without evidence of pump function disturbance were considered as the reference group (non-HF). Patients suffering from long-standing pulmonary diseases or malignity, were excluded from the study.

Histological analyses

The skeletal muscle tissue specimen were processed in 10% phosphate buffered formalin and paraffin embedded. Quantitative microscopy was performed on tissue slices (3 μ m) using specific staining methods and antibody reactions. The following variables were studied: fibre type distribution (both type I and type II fibres), percentage of fat cells, percentage of connective tissue, and the capillary to fibre ratio.

Antibodies and staining techniques

R11D10, an antibody raised against Myosin Heavy Chain type I fibres was used to assess the amount of type I fibres [5]. Control measurements of fibre type distribution were performed by MY-32, which is an antibody raised against MHC type II. Collagen-IV, an antibody raised against type IV collagen, allows selective staining of the basement membrane and was therefore used to count the number of capillaries per fibre [24]. Elastica von Gieson

staining (EvG) was used to define the percentage of fat cells in the skeletal muscle. Sirius-Red staining (SR) was used to quantify the amount of connective tissue [25].

Measurements

At least 100 different fibres and their surrounding capillaries were counted for the capillary to fibre ratio (C/F), and at least 200 fibres were counted for the determination of fibre type distribution. Data of fat and connective tissue percentage were measured on a computerized morphometer Quanti-met 570 (Leica). Measurements of interfascicular fat and interfascicular connective tissue were made on each slice at 6 different areas (1 area = 1.03 mm²) and the mean of these six values was presented.

Statistics

Skeletal muscle characteristics of the chronic-HF group, the mild-HF group and the non-HF group were compared using the Kruskal-Wallis Test. A value of $p < 0.05$ was considered as a significant difference. Values are presented as the mean $\pm$ standard deviation (SD).

Results

Study Subjects

A relatively small biopsy specimen of about 1 gram was obtained peroperatively in six patients with chronic-HF who were submitted for a cardiomyoplasty procedure. Mean age was 57 ± 5 yrs, EF $17 \pm 2\%$, and they were all in NYHA functional class IV. Five of the six were male patients (Table 1).

Biopsies of 27 autopsy patients were collected at the Department of Pathology. Mild-HF was detected in 7 patients (NYHA class II), mean age was 73 ± 8 years, ejection fraction was $41 \pm 15\%$ and 2 of the 7 were male patients (Table 2). In two patients of this group no recent ejection fraction data were available, but based on the

Table 1. Histological analyses of biopsy specimens of the *Latissimus Dorsi* muscle in patients with chronic heart failure. (Chronic-HF).

patient nr.	sex M/F	age	EF %	type I fb. %	type II fb. %	C/F	fat %	con. tissue %
1	M	53	16	29	71	0.7	25	5
2	F	51	16	37	63	0.8	15	6
3	M	57	18	28	72	0.6	9	5
4	M	66	18	30	70	0.8	3	11
5	M	60	16	51	49	0.7	16	2
6	M	57	20	17	83	0.7	7	2
mean		57**	17**	32*	68	0.7	13	5
SD		5	2	11	11	0.1	8	3

EF: ejection fraction, fb.: fibres, C/F: capillary to fibre ratio, con.tissue: connective tissue. *: $p < 0.05$, ** $< p < 0.01$.

clinical information the EF of these patients was judged to be below 50%. Causes of death in the mild-HF group were aorta dissection ($n = 3$), recent MI ($n = 2$), pulmonary embolism and meningitis.

In 10 patients no evidence for heart failure was present and therefore they were enrolled in the non-HF group. Mean age in this group was 74 ± 9 yrs, EF was judged to be normal according to clinical information, and 5 of them were male patients (Table 3). Ten patients were excluded from the study because of malignancy, pulmonary diseases or because of insufficient clinical information on health status.

The mean age of the chronic-HF group was significantly lower than in the other two groups. Although the mild-HF group was selected on a reduced EF value, a significant lower EF was observed in the chronic-HF group ($17 \pm 2\%$ vs $41 \pm 15\%$).

Histological analyses

The percentage of type I fibres, assessed by R11D10 antibody reaction, was significantly lower in chronic-HF, if compared to non-HF ($32\% \pm 11$ vs $52\% \pm 14$), and tended to be lower if compared to the mild-HF group ($32 \pm 11\%$ vs $46 \pm 13\%$) (see tables 1, 2 and 3). This finding was confirmed by the results of fibre type II analyses by MY-32 monoclonal antibody reaction. Representative pictures of the fibre type distribution in latissimus dorsi muscles are shown in figure 1a for chronic-HF, and in figure 1b for non-HF.

The capillary to fibre ratio was not significantly different between the three groups. Whereas this ratio was rather similar in the chronic-HF group (0.7 ± 0.1) the variation was pronounced both in the mild-HF group (1.0 ± 0.3) and in the non-HF group (0.9 ± 0.2) with a range from 0.5 to 1.5.

Figure 2 shows fibres and capillaries by Collagen-IV

antibody reaction in the latissimus dorsi.

There were no differences observed in the percentage of interfascicular fat and connective tissue between the 3 groups. The range in the amount of fat tissue was remarkable in all three groups. A tissue slice showing muscular fat is presented in Figure 3 and a tissue slice stained for connective tissue is shown in Figure 4.

Discussion

At present more than 700 cardiomyoplasty procedures have been performed worldwide, and more applications of skeletal muscle to assist the failing heart are on their way. While numerous clinical and preclinical studies focus on cardiomyoplasty *per se*, the vitality of the LD muscle in this specific group of patients may be of critical importance. Exercise tolerance and fatigue in patients with chronic heart failure were studied by Sullivan [17, 18, 19, 20, 21] and others [3, 12, 13, 23, 26, 27]. It was concluded that not reduced muscle blood flow, but rather an altered skeletal muscle metabolism due to biochemical and morphological changes, was responsible for the earlier onset of the anaerobic threshold in these patients. It is obvious that these intrinsic skeletal muscle changes will affect any application of skeletal muscle for cardiac assist.

Histological analyses by Sullivan [20] and Drexler [3] showed, a significant decrease of type I fibres in chronic heart failure patients (Sullivan normals 52% vs CHF 36%, Drexler normals 55% vs CHF 41%). In contrast, Mancini [12] did not find a difference in muscle fibre type I distribution due to heart failure. The present study reports a significant decrease of slow twitch fibres due to chronic-HF (non-HF 52% vs chronic-HF 32%).

In healthy subjects fibre type distribution shows great variation among different muscle groups, depending on functional demand. Most likely, muscles of the back will

Table 2. Histological analyses of biopsy specimens of the Latissimus Dorsi muscle in patients with mild heart failure. (mild-HF).

patient nr.	sex M/F	age year	EF %	type I fb. %	type II fb. %	C/F	fat %	con. tissue %
1	M	73	57	38	63	0.6	23	2
2	F	72	38	31	69	1.2	26	3
3	F	86	55	41	59	0.6	18	4
4	M	68	28	47	53	1.5	13	7
5	F	67	25	71	29	1.0	18	6
6	F	66	nd	53	47	0.9	6	2
7	F	82	nd	39	61	1.2	30	4
mean		73**	41**	46*	54	1.0	19	4
SD		8	15	13	13	0.3	8	2

EF: ejection fraction, fb.: fibres, C/F: capillary to fibre ratio, con.tissue: connective tissue. nd: not determined *: $p < 0.05$, ** < 0.01 .

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Table 3. Histological analyses of biopsy specimens of the Latissimus Dorsi muscle in patients without heart failure (non-HF).

patient nr.	sex M/F	age year	type I fb. %	type II fb. %	C/F	fat %	con. tissue %
1	M	60	31	69	1.1	11	5
2	F	76	52	48	0.9	8	3
3	M	63	51	49	0.9	3	4
4	M	80	62	38	0.9	14	3
5	F	86	69	31	0.6	29	2
6	M	67	43	57	1.3	10	2
7	F	81	76	23	1.0	25	5
8	M	68	41	59	0.9	2	2
9	F	86	58	42	0.5	23	10
10	F	71	38	62	0.8	19	5
mean		74**	52*	48	0.9	14	4
SD		9	14	14	0.2	9	2

Note: different from the patients in the other groups, the ejection fraction in the non-HF group is not known, but assumed to be normal based on clinical information.

fb.: fibres. C/F: capillary to fibre ratio, con.tissue: connective tissue.

* $p < 0.05$, ** $p < 0.01$.

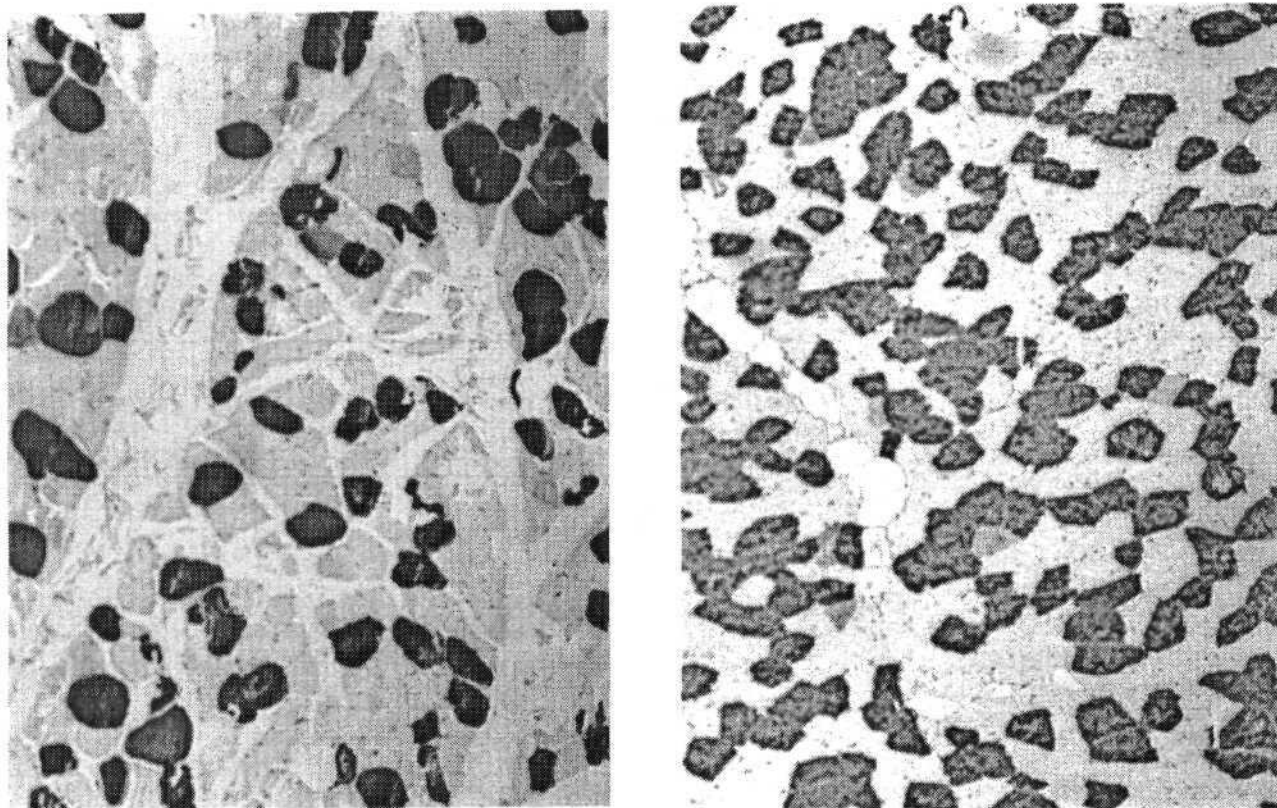


Figure 1. Composition of muscle fibres in the Latissimus Dorsi muscle of patients with chronic heart failure (left, 29% type I fibres) and in controls (right, 50% Type I fibres). Antibody reaction for type I fibres with R11D10. Type I fibres appear dark; type II fibres appear white.

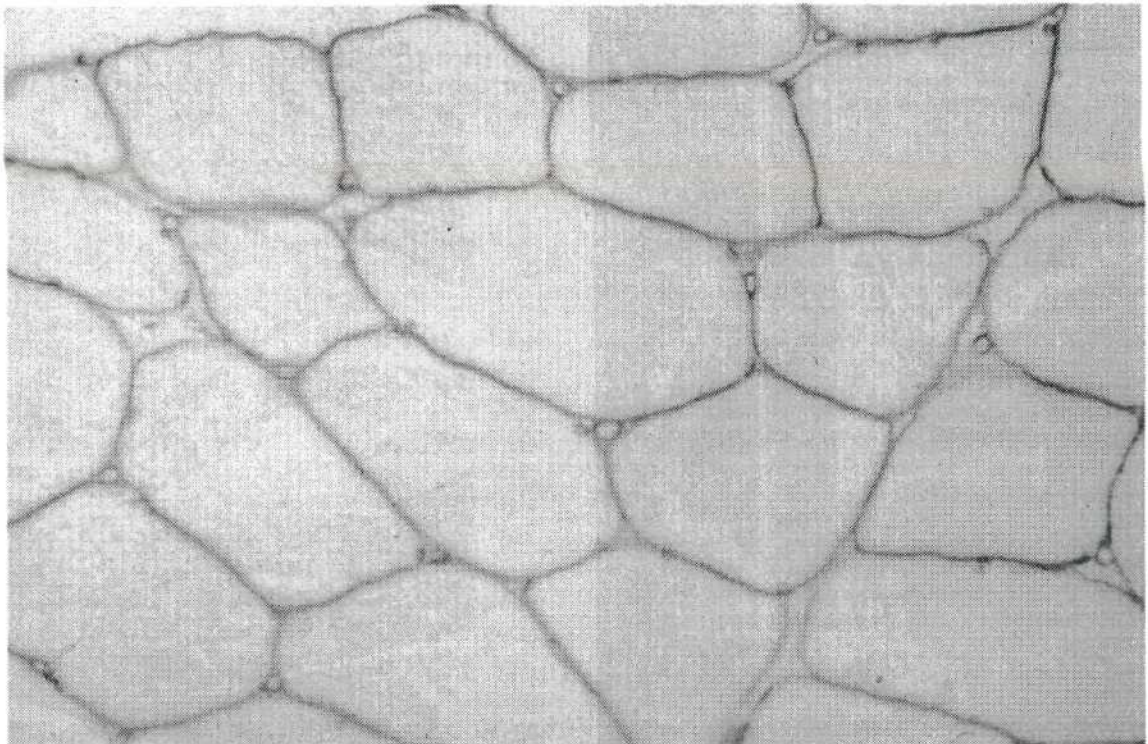


Figure 2. Antibody reaction with Collagen-IV to stain the basement membrane showing both capillaries (C) and muscle fibres (F) of the Latissimus Dorsi muscle.

be influenced less from inactivity than the muscles of the legs. On the other hand, if chronic heart failure is accompanied by a systematic decrease of muscle vitality one would expect to observe the same deterioration in all muscles. Sullivan and Drexler obtained biopsy specimen of the vastus lateralis muscle, Mancini analyzed muscle specimen of the gastrocnemius, while our data represent values of the LD muscle. In skeletal muscle studies usually muscles of the lower limbs are selected. However, we took biopsies of the LD muscle because of its application in cardiomyoplasty.

Also Johnson [6] and Scelsi [15] analyzed the LD muscle. Johnson found a mean percentage of 50.5% type I fibres in healthy young men (aged 18 - 30 years). Scelsi examined the LD muscle of sedentary male subjects (aged 40 - 65 years) and reported a percentage of 50% type I fibres.

These data correspond to our findings of 52% type I fibres in non-HF patients, despite the fact that our control subjects had a significant older age, which indicates that fibre type distribution of the LD muscle does not change per se due to aging. This is in line with the finding of Coggan and colleagues [2] who showed that the percentage of type I, IIa and IIb fibres in lower extremity muscles did not differ with age.

The number of capillaries in skeletal muscle tissue is generally related to the intensity of exercise and hence of muscle metabolism, and can be expressed in relation to muscle surface, muscle fibres, or to fibre type. However, a

large variation is observed in data presenting the capillary density using the capillary to fibre ratio. Table 4 shows the C/F ratio observed in different studies, using different skeletal muscles [1, 12, 15, 20]. The variation in C/F, both in normal subjects and in patients can be explained on the one hand by the fibre type distribution of a muscle group, and on the other hand by the definition used for determining capillary to fibre ratio. Fluctuation in this ratio can occur, because capillaries can belong to more than one fibre, which means that counting only a few fibres per area increases the C/F ratio. In the present study we observed no decrease of the capillary to fibre ratio in chronic-HF.

The amount of connective tissue measured in the three groups was rather similar with a low variation and mean percentages of 4% to 5%. Collagen fibres are a prerequisite for myosin and actin filaments to return to their original orientation after contraction. However, an excessive growth of collagen fibres in between muscle fibres, increases the stiffness of the muscle and therefore reduces relaxation. MacDougall [10] studied three groups of healthy men who were subjected to different degrees of training. He concluded that in all groups the amount of non-contractile tissue was extremely consistent and was about 13% of the tissue sample after training. Of this 13% about 6% was identified as collagen (connective tissue), which is in line with the findings of the present study.

The appearance of fat cells in skeletal muscle tissue is not often described. Jones [7] reported fat percentages in both the quadriceps and the calf muscle to be less than 5% of



Figure 3. Histological appearance of the Latissimus Dorsi in a chronic-HF patient showing the infiltration of fat cells. Elastine von Gieson stain.

muscle tissue. However, in these same muscles relatively high percentages of connective tissue were observed (11.5%). We found in our chronic-HF group a mean fat percentage of 13%.

In the present study this percentage did not differ from mild-HF and control subjects. Scelsi [15] measured in the LD muscle a mean percentage of 19% fat (range 12-25%) in sedentary autopsy patients. This percentage corresponds well to our data. Grindrod [4] has pointed out that more than 20% fat in skeletal muscle tissue will decrease the muscle contractile force with more than 35%, and even smaller amounts of fat infiltration in skeletal muscle are accompanied by loss of contractile force. This item is important for aorto- and cardiomyoplasty, as optimal power generation is required for cardiac assist.

Conclusion

The morphology of the LD muscle of patients with chronic heart failure is significantly altered. The reduction of type I fibres is a disadvantage for aorto- and cardiomyoplasty were LD muscle conditioning should change the muscle into a predominantly (> 90%) type I fibre muscle.

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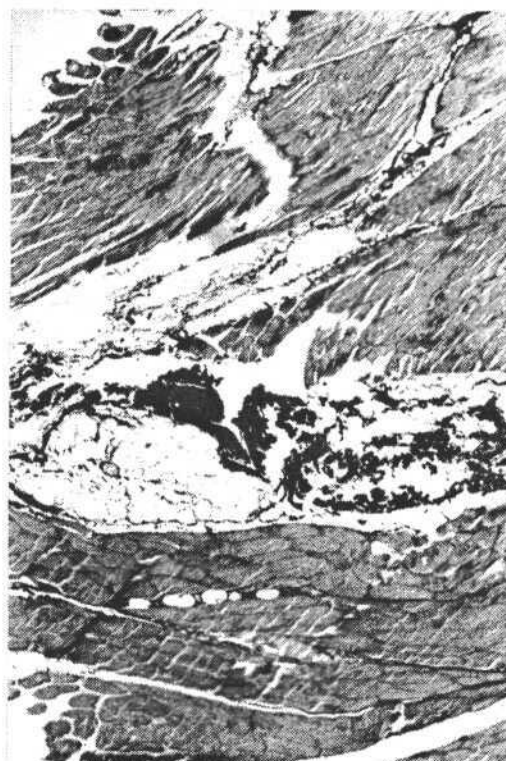


Figure 4. Connective tissue in the Latissimus Dorsi muscle of a chronic-HF patient. Sirius Red-stain.

Table 4. Survey of capillary to fibre ratio (C/F) in skeletal muscle of patients with chronic heart failure (CHF) and healthy individuals (non-HF). Data are presented as mean $\pm$ SD.

Reference	patients group	examined muscle	C/F
Sullivan (1990)	CHF non-CHF	Vastus Lateralis	$4.0 \pm 0.7^{\#}$ $4.8 \pm 0.4^{\#}$
Mancini (1989)	CHF non-CHF	Gastrocnemius	1.85 ± 0.35 1.67 ± 0.25
Carry (1986)	young adults	Vastus Lateralis	1.47 ± 0.09
Scelsi (1990)	sedentary patients (autopsy)	Latissimus Dorsi	1.20 ± 0.08

C/F: capillary to fibre ratio. #: capillary to type I fibre ratio

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