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Myopathy in CRPS-I: Disuse or neurogenic?

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ABSTRACT

The diagnosis Complex Regional Pain Syndrome type I (CRPS-I) is based on clinical symptoms, including motor symptoms. Histological changes in muscle tissue may be present in the chronic phase of CRPS-I. Aim of this study was to analyze skeletal muscle tissue from amputated limbs of patients with CRPS-I, in order to gain more insight in factors that may play a role in changes in muscles in CRPS-I. These changes may be helpful in clarifying the pathophysiology of CRPS-I. Fourteen patients with therapy resistant and longstanding CRPS-I, underwent an amputation of the affected limb. In all patients histological analysis showed extensive changes in muscle tissue, such as fatty degeneration, fibre atrophy and nuclear clumping, which was not related to duration of CRPS-I prior to amputation. In all muscles affected, both type 1 and type 2 fibre atrophy was found, without selective type 2 fibre atrophy. In four patients, type grouping was observed, indicating a sequence of denervation and reinnervation of muscle tissue. In two patients even large group atrophy was present, suggesting new denervation after reinnervation. Comparison between subgroups in arms and legs showed no difference in the number of changes in muscle tissue. Intrinsic and extrinsic muscles were affected equally. Our findings show that in the chronic phase of CRPS-I extensive changes can be seen in muscle tissue, not related to duration of CRPS-I symptoms. Signs of neurogenic myopathy were present in five patients.

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1. Introduction

The term Complex Regional Pain Syndrome type I (CRPS-I), to describe a syndrome previously known under the name of Reflex Sympathetic Dystrophy, was recommended in 1994 by the International Association for the Study of Pain (IASP) (Merskey and Bogduk, 1994; Stanton-Hicks et al., 1995). The term is based on clinical symptoms including sympathetic, somatomotor and somatosensory symptoms. The pathophysiology of CRPS-I is still basically unknown, although peripheral, autonomic and central mechanisms are likely involved (Raja and Grabow, 2002; Jänig and Baron, 2003). To facilitate clinical research and permit specific targeting of treatments, two subtypes of CRPS have been suggested by the IASP (Merskey and Bogduk, 1994; Stanton-Hicks et al., 1995). CRPS type I may develop after trauma without nerve lesion, type II develops after trauma with nerve lesion. Up till now, the description of subtypes has as yet not been helpful in clarifying pathophysiology or targeting treatment.

In many patients with CRPS-I motor symptoms are found, such as muscle weakness, muscle atrophy and reduced range of motion (Veldman et al., 1993; Raja and Grabow, 2002; Jänig and Baron, 2003). Furthermore, pain and a neglect-like syndrome may lead to disuse of the limb with even more disabling consequences (Galer and Jensen, 1999). Observing the motor symptoms in chronic CRPS-I, changes in muscle tissue may be expected. Reports on histological analysis of skeletal muscle tissue in CRPS-I are scarce for two reasons. Biopsies to obtain tissue from an affected limb are often painful and a widespread belief exists that biopsies may deteriorate clinical symptoms of CRPS-I. Therefore histological analysis of tissues from amputated limbs is preferred. Studies on histological analysis of tissue in CRPS-I show results varying from myopathy and microangiopathy in muscle tissue (Tilman et al., 1990; van der Laan et al., 1998) to neuropathologic changes in skin tissue (Albrecht et al., 2006; Oaklander et al., 2006). These study results indicate that further histopathological investigation of amputated limbs may be helpful in clarifying the pathophysiology of CRPS-I.

Based on metabolic properties, skeletal muscle fibres can be distinguished in: Type 1/slow oxidative/red, Type 2A/fast oxidative/red, Type 2B/fast glycolytic/white and Type 2C/undifferentiated.

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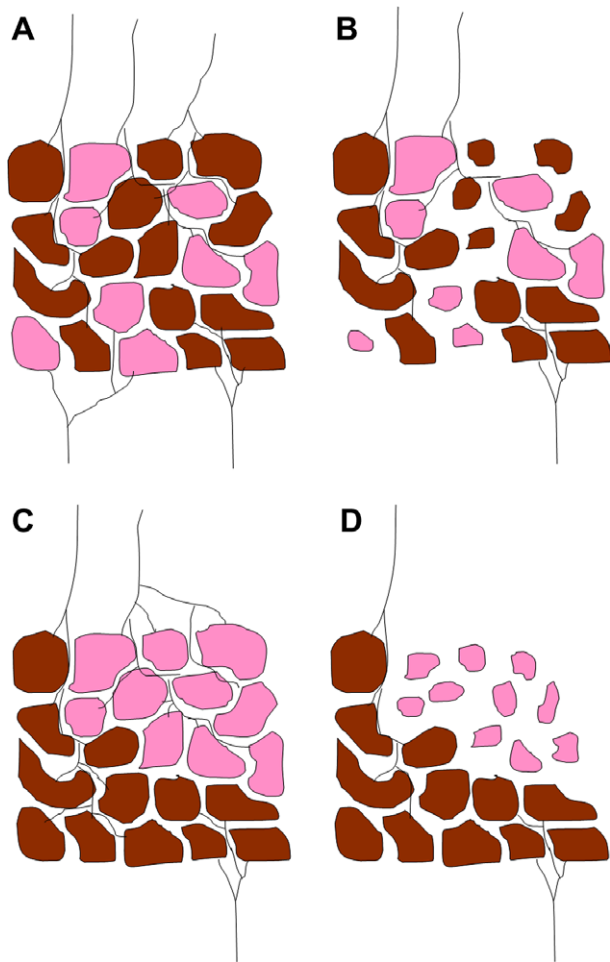


Fig. 1. (1A), (1B), (1C), (1D) Scheme of denervation patterns. (1A) Normal checkerboard pattern of type 1 (brown) and type 2 fibres (pink). (1B) Denervation, showing atrophy of both type 1 and type 2 fibres. (1C) Reinnervation showing type grouping of muscle fibres. (1D) New denervation, showing large group atrophy.

Using ATP-ase enzyme-histochemical stainings at different pH, the different skeletal muscle fibres can be evaluated under the microscope. An ATP-ase staining at pH 4.3 will result in dark Type 1 and light Type 2A and 2B fibres. All muscle fibres in a specific motor unit are of the same metabolic type (Dubowitz and Pearce, 1960). Disuse causes a decrease in size of muscle fibres (atrophy), which affects type 2 fibres more than type 1 fibres (Dubowitz et al., 2007). Muscle fibres will also atrophy when deprived of the trophic influence of their nerve (denervation), which affects both type 1 and type 2 fibres (Fig. 1A and B). Reinnervation of muscle tissue leads to so-called type grouping (Fig. 1C), indicating that small muscle fibres and large muscle fibres tend to cluster. If denervation occurs again, large group atrophy may be observed (Dubowitz et al., 2007) (Fig. 1D). These typical atrophy patterns can be used in studying the pathophysiology of chronic CRPS-I myopathy.

Aim of this study was to analyze skeletal muscle tissue from amputated limbs of patients with chronic CRPS-I, in order to get insight into the pathophysiology of changes of muscle tissue in CRPS-I.

2. Methods

2.1. Patients

Patients with CRPS-I of a limb with serious infections in that limb, a complete afunctional limb and requesting an amputation

of the affected limb were referred to the Centre for Rehabilitation of the University Medical Centre Groningen. CRPS-I was diagnosed in all patients according to IASP criteria (Merskey and Bogduk, 1994). In the period of May 2000 to June 2005 14 patients underwent amputation of one limb.

Medical history was retrieved from the medical records. The following data were collected: gender, co-morbidity, affected limb, inciting event, indication for amputation, CRPS-I duration at amputation, level of amputation, age at amputation and follow-up data about recurrence of CRPS-I. Duration of CRPS-I was calculated from the moment the patients met diagnostic criteria for the first time. Both patients with CRPS-I of the upper and lower extremity were included. Patients with a medical history of nerve lesions, nerve disease or diabetes were excluded.

2.2. Muscle tissue

Patients were asked for permission to perform histological analysis of the amputated limb. All patients or their care givers, in case of children, gave written informed consent prior to amputation. In 6 patients sampling of muscle tissue was performed according to the written protocol (Appendix A). In 8 patients 3 or more muscle samples were obtained with at least one intrinsic and one extrinsic muscle from different innervating nerves.

Muscle tissue was stained for light microscopic investigations; muscle tissue for paraffin embedding and frozen tissue for enzyme-histochemistry. ATP-ase staining was performed for fibre typing. From frozen muscle tissue 5 μ m sections were cut and dried for 30 min followed by pre-incubation with a Veronal-Acetate/HCl buffer at pH 4.3 for 5 min. Thereafter incubation was performed with the same buffer containing 30 mg of ATP at 37 °C for 45 min. Thereafter a series of rinsing steps were performed (1% CaCl_2 -Formol 2 \times 5 min, 2% Kobaltchloride 3 min, aqua-dest 1 min, 2% Sodiumsulfide for 10 s and again aqua-dest), followed by a counterstaining with eosin. This enzyme-histochemical staining procedure resulted in dark-brown type 1 fibres, pink type 2A and 2B fibres and light brown 2C (undifferentiated) fibres.

The enzyme-histochemical stainings of the muscle tissue were evaluated for the presence of type grouping, indicating a sequence of denervation and reinnervation of the muscle tissue. Furthermore, the distribution of atrophy amongst the various fibre types was evaluated. Selective atrophy of type 2 fibres would point more in the direction of disuse, whereas a combined atrophy of type 1 and 2 fibres would point more in the direction of a neurogenic myopathy (Fig. 1A–D).

Tissue characteristics assessed were: fatty degeneration, connective tissue formation, fibre atrophy, fibre diameter variance, nuclear clumping and large group atrophy (Fig. 2A–C). In healthy muscle tissue these characteristics are not present. Classification of severity of tissue changes was self-designed, as no validated classification is available in the literature. The following classification was made: fatty degeneration was ranked into: 0 = absent; 1 = present, connective tissue formation into: 0 = absent; 1 = present, fibre diameter variance into: 0 = none; 1 = little; 2 = intermediate; 3 = severe, fibre atrophy into: 0 = none; 1 = focal; 2 = diffuse, nuclear clumping into: 0 = none; 1 = focal; 2 = diffuse and large group atrophy into 0 = absent; 1 = present. Large group atrophy indicates to a sequence of denervation, reinnervation and again denervation, as earlier described in the introduction.

All specimens were reviewed by the last author. The last author is a neuropathologist and was blinded to the clinical history. The first author was present in the same session to process the findings. A selection of the samples was reviewed a second time by both authors, obtaining the same results.

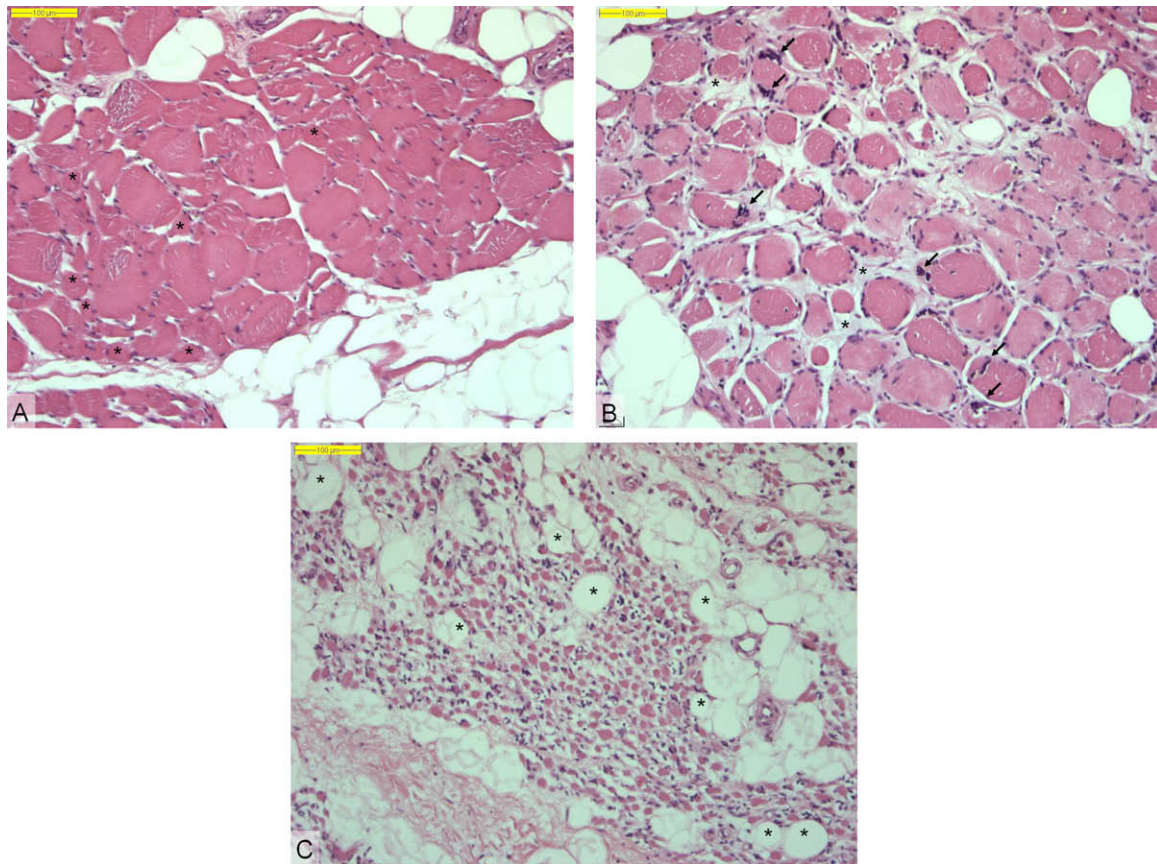


Fig. 2. (2A), (2B), (2C) Photomicrographs of transverse sections of various skeletal muscle samples, ranging from minimally to severely affected. The bars in the figures represent 100 µm. (2A) shows an increase in the variance of the muscle fibre diameter. Relatively atrophic muscle fibres are indicated with an asterisk. (2B) shows clumping of pyknotic nuclei (arrows) and some fibrosis (asterisks) pointing at more chronic atrophy. (2C) shows diffuse atrophy with nuclear clumping and fatty changes (asterisks).

2.3. Analysis

Descriptive data analysis of tissue characteristics was conducted using SPSS for Windows version 14. Different tissue changes were dichotomized for each sample into absent or present. Per sample the number of tissue changes was summed into the total number of tissue changes present. Duration of CRPS-I was correlated with the total number of tissue changes to analyze a possible relationship between duration of CRPS-I and the total number of tissue changes in the samples. Additionally nonparametric tests (Mann-Whitney U) were used to analyze differences in total number of tissue changes between samples from arms and legs and between samples from intrinsic and extrinsic muscles.

3. Results

3.1. Patients

The group of patients consisted of 2 males and 12 females, with a median age at amputation of 39.5 years (IQR 29.5; 47.2), and median duration of CRPS-I of 4.5 years (IQR 2.0; 8.0) (Table 1). In all patients the limb was afunctional for more than 2 years prior to amputation because of pain, oedema, muscle weakness and reduced range of motion. In 11 patients the symptoms of CRPS-I were preceded by minor trauma or minor surgery. In 3 patients there was no apparent inciting event. Amputation was performed in 3 arms and 11 legs (Table 1). All patients had received several treatments before amputation, such as physiotherapy, oxygen free radical scavengers, mannitol infusions and various drugs. Seven patients were treated with sympathetic nervous blockade or sym-

pathectomy (patients 2, 3, 4, 8, 9, 11 and 13) (Table 1). In 2 patients (patients 8 and 10) more than one limb was affected by CRPS-I (respectively, 4 and 2 limbs), but only one limb was amputated. Follow-up data after amputation were present for all patients, with a median follow-up duration of 16.5 months (IQR 5.6; 39.0). Neither signs of recurrence of CRPS-I were present in the stumps, nor signs of a newly developed CRPS-I in another part of the body.

3.2. Muscle tissue

In all amputated limbs changes of skeletal muscle tissue were observed (Table 2, Figs. 2A–C, and 3A and B). In a total of 55 samples only 2 samples were normal. These samples were taken from extrinsic muscles of two different patients. Fatty degeneration was present in half of the samples, and connective tissue formation in almost none of the samples. Large group atrophy was present in 4 samples of patient 5 and 14. Patient 5 was female, 16 years of age, with no apparent inciting event for CRPS-I. The duration of symptoms till amputation was at least 2 years. Patient 14 was male, 49 years of age, with CRPS-I after a calcanean tendon rupture; the duration of symptoms till amputation was at least 4 years. In both patients muscle tissue changes were more severe than in the other patients. In the two patients with more than one limb affected, tissue changes did not differ in severity from those of other patients. A longer duration between diagnosis of CRPS-I and amputation did not significantly correlate with the number of tissue changes in the specimens ($\rho = -0.15$, $p = 0.26$). The number of tissue changes did not differ between samples from intrinsic muscles (mean: 3.5; SD: 1.0) and those from extrinsic muscles (mean: 3.2; SD: 1.3) ($p = 0.34$). The number of tissue

Table 1
Characteristics of the CRPS-I patient group ($n = 14$)

Subject	Age at amputation (years)	Gender	Duration CRPS I prior to amputation (years)	Limb	Number of samples	Level of amputation	Most important indications for amputation	Inciting event	Treatment prior to amputation	Duration of follow-up (months)
1	47	F	9	Leg	3	KEA	Pain, rROM, afunfunctional	None	PT, OFRS, VD	39
2	45	F	7	Arm	5	THA	Pain, infections, rROM, afunfunctional	Minor trauma, lifting music instrument	PT, OFRS, VD, MI, SB, ST	3
3	41	F	2	Leg	4	TTA	Ulceration, necrosis, afunfunctional	Minor trauma followed by arthroscopy knee	PT, OFRS, VD, MI, SB	44
4	16	F	4	Leg	4	KEA	Pain, rROM, afunfunctional	Sprained ankle	PT, OFRS, VD, SB, ST	41
5	25	F	2	Leg	3	TFA	Pain, ulceration, afunfunctional	None	PT, OFRS, VD	17
6	52	F	2	Leg	4	TTA	Pain, rROM, afunfunctional	Operative correction foot	PT, OFRS, VD, MI	2
7	48	F	12	Leg	4	TFA	Pain, afunfunctional	Arthroscopy knee	PT, VD, MI	23
8	32	F	6	Arm	4	THA	Pain, ulceration, afunfunctional	Short entrapment of the hand without apparent injury	PT, OFRS, VD, MI, SB	0.5
9	38	F	3	Leg	4	KEA	Pain, ulceration, afunfunctional	HNP operation	PT, OFRS, VD, MI, SB, ST	39
10	31	F	8	Leg	4	KEA	Ulceration, infections, afunfunctional	Minor trauma	PT, OFRS, VD, MI	6
11	36	F	5	Leg	4	TFA	Ulceration, afunfunctional	Sprained ankle	PT, VD, ST	16
12	23	M	2	Arm	4	AF	Ulceration	Amputation MCP dig III	PT, VD, MI	4.5
13	42	F	8	Leg	4	TTA	Pain, afunfunctional	Minor trauma	PT, OFRS, VD, MI, SB, ST	13
14	49	M	4	Leg	4	KEA	Pain, afunfunctional	Calcanean tendon rupture	PT, VD	10

F = female, M = male, CRPS-I = complex regional pain syndrome type I, TFA = transfemoral amputation, KEA = exarticulation of the knee, TTA = transtibial amputation, THA = transhumeral amputation, AF = amputation of forearm, rROM = reduced range of motion, afunfunctional = afunfunctional limb, HNP = hernia nuclei pulposi, MCP = metacarpophalangeal, dig = digit, PT = physiotherapy OFRS = oxygen free radical scavengers, VD = various drugs, MI = mannitol infusion, SB = sympathetic block, ST = sympathectomy.

Table 2
Changes in muscle tissue samples of 14 amputated limbs

Category		Arms (n of samples = 13) % (n)	Legs (n of samples = 42) % (n)	Extrinsic muscle (n of samples = 28) % (n)	Intrinsic muscle (n of samples = 27) % (n)	All (n of samples = 55) % (n)
1. Fatty degeneration	0 = absent	38 (5)	50 (21)	50 (14)	44 (12)	47 (26)
	1 = present	62 (8)	50 (21)	50 (14)	56 (15)	53 (29)
2. Connective tissue formation	0 = absent	100 (13)	93 (39)	96 (27)	93 (25)	95 (52)
	1 = present	0 (0)	7 (3)	4 (1)	7 (2)	5 (3)
3. Fibre diameter variance	0 = none	0 (0)	5 (2)	7 (2)	0 (0)	4 (2)
	1 = little	92 (12)	69 (29)	68 (19)	81 (22)	74 (41)
	2 = intermediate	8 (1)	21 (9)	21 (6)	15 (4)	18 (10)
	3 = severe	0 (0)	5 (2)	4 (1)	4 (1)	4 (2)
4. Fibre atrophy	0 = none	0 (0)	5 (2)	7 (2)	0 (0)	4 (2)
	1 = focal	100 (13)	69 (29)	71 (20)	81 (22)	76 (42)
	2 = diffuse	0 (0)	26 (11)	21 (6)	19 (5)	20 (11)
5. Nuclear clumping	0 = none	15 (2)	29 (12)	28 (8)	22 (6)	26 (14)
	1 = focal	85 (11)	50 (21)	54 (15)	63 (17)	58 (32)
	2 = diffuse	0 (0)	21 (9)	18 (5)	15 (4)	16 (9)
6. Large group atrophy	0 = absent	100 (13)	90 (38)	93 (26)	93 (25)	93 (51)
	1 = present	0 (0)	10 (4)	7 (2)	7 (2)	7 (4)
Number of categories containing tissue changes	0	0 (0)	5 (2)	7 (2)	0 (0)	4 (2)
	1–2	15 (2)	19 (8)	21 (6)	15 (4)	18 (10)
	3–4	85 (11)	64 (27)	64 (18)	74 (20)	69 (38)
	5–6	0 (0)	12 (5)	7 (2)	11 (3)	9 (5)

Percentages are column percentages; due to rounding of sum percentages may not equal 100%.

changes did not differ between specimens taken from arms (mean: 3.5; SD: 0.8) and those from legs (mean: 3.3; SD: 1.3) ($p = 0.64$).

Concerning the ATP-ase enzyme-histochemistry, in all the affected muscles described above, both type 1 and type 2 fibres showed atrophy, without predilection for one or the other. In 4 pa-

tients clear signs of type grouping could be observed, indicating a sequence of denervation and reinnervation (i.e. neuronal damage). In patients 1, 10 and 14 the type grouping was found in the m. adductor hallucis. In patient 2 the type grouping was found in both the m. extensor digitorum and m. flexor digiti minimi.

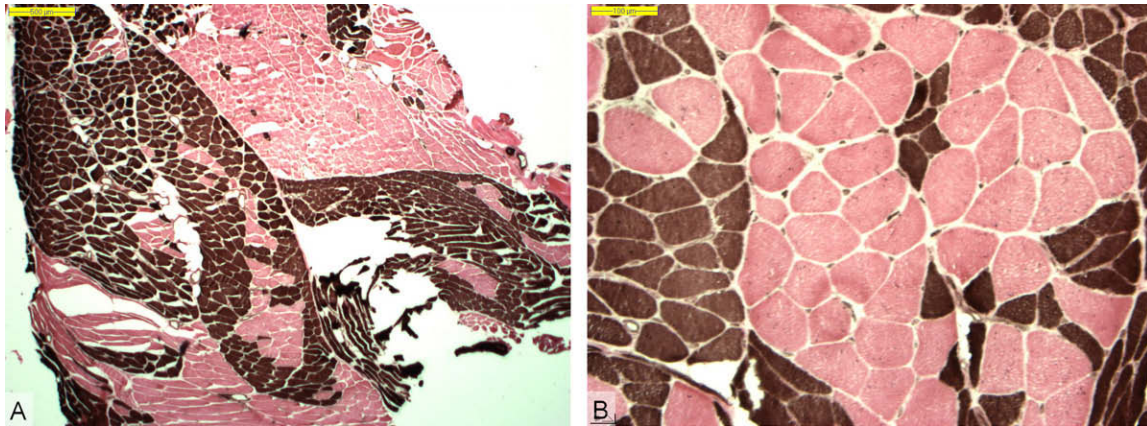


Fig. 3. (3A) and (3B) Photographs of transverse sections of skeletal muscle samples with enzyme-histochemical staining for evaluation of the presence of type grouping and the distribution of atrophy amongst type 1 and type 2 fibres. In both figures atrophy of both type 1 and type 2 fibres is present. The bars represent 500 µm in 3A and 100 µm in 3B.

4. Discussion

Extensive changes in muscle tissue were present in patients with longstanding CRPS-I. The changes were similar in all patients. The investigated muscle tissue belonged to patients with CRPS-I with an immobilized, afunctional limb for more than 2 years. Prior to this study, a correlation between duration of CRPS-I and the number of muscle tissue changes was expected, a result of a longer period of disuse. However, in the current study no such relationship was found.

CRPS described by the IASP criteria differentiates between two subtypes, type I and II, according to the presence of nerve lesions after trauma. In the current study, both type 1 and type 2 fibre atrophy was found in all the affected muscles. Would disuse have been the cause of muscle changes, type 2 fibres would have atrophied more selectively. In 4 out of 14 patients, type grouping was observed, indicating a sequence of denervation and reinnervation of muscle tissue. In 2 out of 14 patients even large group atrophy was present, suggesting new denervation after reinnervation (in one of these patients type grouping was also present). When nerve damage plays a role in CRPS-I, one would also expect to see intrinsic muscles being more affected than extrinsic muscles and legs being more affected than arms, because of the difference in nerve length and nerve vulnerability. Although type grouping occurred more often in the intrinsic muscle tissue of the foot, no difference in the number of changes on routine histology was found between samples from arms and those from legs. In the medical history of the patient with large group atrophy, neither for the patients with type grouping nor for the patient with both large group atrophy and type grouping a nerve lesion was described. According to CRPS-I criteria there should not be nerve damage present. The initiating events of CRPS-I in these patients consisted of minor trauma or were not apparent, without reason for nerve damage. The question then arises if nerve damage can be seen in samples of nerve tissue and, if so, what causes this nerve damage since no apparent nerve damage was recorded in the history of these patients. Perhaps, in the chronic phase of CRPS-I, other factors than the initial inciting event may cause denervation. All patients received several types of treatment before amputation (Table 1). However, these treatments, including sympathectomy, are not known for causing motor nerve damage and therefore considered not relevant to our findings.

Perhaps the circulation in or to the nerve is hampered by the chronic existence of oedema or microangiopathy. Additionally inflammation of blood vessels or nerve tissue also may play a role in denervation observed in the two patients. Trophic changes are more frequently present in patients with CRPS-I in the chronic

phase, compared to patients with CRPS-I in earlier phases (Veldman et al., 1993). However, of these patients with chronic CRPS-I, more than half did not show clinical signs of atrophy (Veldman et al., 1993). In the current study all patients with longstanding CRPS-I showed clinical signs of muscle atrophy.

In an histological analysis of muscle tissue of the gastrocnemius and the soleus muscle of amputated lower limbs in 8 patients because of CRPS-I, a variety of changes was seen (van der Laan et al., 1998). In the muscles a decrease of type 1 fibres was found, as well as an increase of lipofuscin and atrophic fibres. Capillaries showed microangiopathy similar to the histological abnormalities found in skeletal muscle of diabetic patients which seem to be related to ischemic conditions. In an electron microscopical evaluation of muscle biopsies of gastrocnemius muscle in patients with chronic CRPS-I both neuropathic and myogenic muscle changes were seen, with signs for oxidative stress. No signs of cellular inflammation occurred (Tilman et al., 1990).

In the current study 14 patients were included with samples from different subgroups of muscles. By using several samples we tried to find specific indications for denervation. Both van der Laan and Tilman describe changes in cytological architecture, but do not specifically describe atrophy patterns that may point to neurogenic myopathy (Tilman et al., 1990; van der Laan et al., 1998).

A limitation of the study is that we were not able to compare our tissue samples with healthy tissue samples. In our hospital limbs may be amputated because of atherosclerotic or neoplastic changes; the latter mostly after (local) chemotherapy. Tissues from these limbs may not suffice as controls.

On clinical presentation to our centre all patients had not used their affected limb for over two years and all presented with contractures and pain. To discern paralysis from disuse solely on clinical presentation was therefore, not possible. Moreover, because of the heterogeneity of the symptoms of CRPS-I it was very difficult to get a homogenous population in terms of clinical symptoms. We therefore chose only to describe clinical symptoms and not to use them as parameters in relation to the histological findings. To be able to compare muscle tissue in this group of patients sampling was performed according to protocol.

Results of nerve conduction studies or EMG prior to amputation were not used in our study. In half of the patients these studies were performed in an earlier stage as an adjuvant method in diagnosis, however with very heterogeneous methods and results. Moreover, nerve conduction studies and EMG in CRPS-I are often very painful and, therefore, difficult to use as a method to assess nerve damage.

Our findings show that in the chronic phase of CRPS-I extensive changes are present in muscle tissue, which are not related to the

duration of CRPS-I. No differences were seen in different subgroups of muscles (i.e. intrinsic vs. extrinsic; arm vs. leg). The most important finding was that in 5 patients signs of denervation were found pointing at a nerve lesion. Factors that may play a role in changes of muscle tissue can be neuropathic or vascular in origin, in which disuse may be a less important factor, which is underlined by the absence of selective type 2 fibre atrophy. For the future we are planning to investigate human nerve tissue and vascular tissue as a following step to clarify pathophysiology in CRPS-I.

Appendix A

Protocol for obtaining samples of various tissues in amputated limbs of CRPS-I patients

	<i>Method of sampling</i>
Arm	<ul style="list-style-type: none"> – intrinsic hand muscles: m. adductor pollicis (ulnar nerve), m. abductor pollicis (median nerve) – extrinsic hand muscles: m. flexor digitorum superficialis (median nerve), m. flexor carpi ulnaris (ulnar nerve), m. extensor carpi radialis (radial nerve)
Leg	<ul style="list-style-type: none"> – intrinsic foot muscles: m. adductor hallucis (tibial nerve) m. extensor digitorum brevis (peroneal nerve) – extrinsic foot muscles: m. tibialis anterior (peroneal nerve), m. gastrocnemius (tibial nerve)
	<i>Method of investigation</i>
	– muscle tissue for paraffin embedding and frozen tissue for enzyme-histochemistry (ATP-ase for fibre typing)

m. = muscle, ATP = adenotriphosphate.

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