Peripheral nerve pathology in patients with severely affected complex regional pain syndrome type I

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Complex regional pain syndrome type I (CRPS-I) is a chronic pain syndrome with no clinical evidence of nerve injury; however, recently, changes in muscle tissue have been found in case of CRPS-I. Our aim was to search for histological changes in peripheral nerves of amputated limbs from patients with therapy-resistant CRPS-I that could justify muscle tissue changes. Fifteen patients with CRPS-I (duration > 1 year) were included. Multiple nerve samples were taken from upper ($n = 4$) and lower ($n = 11$) amputated limbs. Histological changes (signs of nerve fiber loss and regeneration), fiber diameters, fiber diameter distribution, and fiber density were studied through microscopy and morphometry. Samples from three healthy sural nerves were used as control data as well as data from the literature. All patients (93% of tissue samples) showed histological signs of nerve fiber loss and fiber regeneration, varying in severity. No specific preference was found for any nerve or the location within the nerve. Sural nerves showed loss of especially larger nerve fibers (>12 μm) in comparison with control data. Sympathectomy did not influence this finding. The morphometric results of the other nerves are more difficult to interpret because of the absence of good-quality control data from the literature. However, the percentages of nerve fibers greater than 12 μm seem to lie within the normal range. Besides the known pathology of thin nerve fibers innervating the skin or blood vessels in CRPS-I, this study also shows pathological changes more proximal in the nerves, especially in the sural nerve. ***International Journal of Rehabilitation Research*** **00:000–000 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Background

In complex regional pain syndrome (CRPS), two types are distinguished: CRPS type I (CRPS-I) is considered a syndrome without nerve injury and CRPS type II (CRPS-II) is considered to develop after nerve injury (Stanton-Hicks *et al.*, 1995). CRPS-I is characterized by (extreme) pain in a distal part of a limb, motor impairment, and autonomic dysfunction. The intensity of the pain is disproportionate to the inciting event. The pathophysiology of CRPS-I is unknown, although several hypotheses have been proposed such as neurogenic inflammation, endothelial dysfunction, and pathological sympathetic–afferent coupling (Birklein *et al.*, 2001; Weber *et al.*, 2001; Baron *et al.*, 2002; Schattschneider *et al.*, 2006). The presence of oxidative stress, hypoxia, and inflammatory processes together with neuropathic pain mechanisms are currently the leading hypotheses (Coderre and Bennett, 2008; Marinus *et al.*, 2011).

Changes in muscle tissue have been suggested on the basis of motor impairment present in CRPS-I (Veldman *et al.*, 1993; Jänig and Baron, 2002; Raja and Grabow, 2002). Results from research on skin biopsies in patients with CRPS-I suggest nerve damage in CRPS-I, like CRPS-II, but assumed to be predominantly affecting small diameter fibers (Albrecht *et al.*, 2006; Oaklander *et al.*, 2006). Small fiber neuropathy can explain the pain and autonomic dysfunction (Albrecht *et al.*, 2006; Oaklander *et al.*, 2006). According to Van der Laan *et al.* (1998a, 1998b), myelinated fibers did not show consistent abnormalities. However, in four of eight patients in their study, a decrease in myelinated fiber density was found (Van der Laan *et al.*, 1998a). A decrease in especially larger myelinated fibers (>9 μm) was found in an animal model (Guilbaud *et al.*, 1993). Pathology in large nerve fibers could explain the recent findings in CRPS-I muscle tissue showing signs of denervation, reinnervation, and again denervation (Hulsman *et al.*, 2009). On the basis of those results, we expect larger nerve fibers to be affected in CRPS-I too. Because of the differences between the results of muscle tissue analyses of patients amputated because of CRPS-I and the results from the literature, the aim of this study was to analyze samples of peripheral nerve tissue of patients amputated because of CRPS-I.

Methods

Patients

In very few patients with CRPS-I, severe complications such as infections, ulcers, chronic edema, and a dysfunctional limb

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may develop. These complications are difficult to treat (Van der Laan *et al.*, 1998b; De Mos *et al.*, 2009). Amputation of the limb affected by CRPS-I is not a common procedure and whether or not it should be performed is debatable (Bodde *et al.*, 2011). Amputation of the affected limb is sometimes a patient's last resort in an attempt to restore quality of life (Hohendorff *et al.*, 2011; Krans-Schreuder *et al.*, 2012).

Between May 2000 and March 2007, 18 patients with longstanding and therapy-resistant CRPS-I underwent an amputation at the University Medical Center Groningen, the Netherlands. CRPS-I was diagnosed according to IASP criteria (Stanton-Hicks *et al.*, 1995). Re-evaluation of the patient files showed that CRPS-I would have been diagnosed according to the Budapest criteria as well (Harden *et al.*, 2007; Harden *et al.*, 2010). All patients ($n = 18$) requested amputation because of severe pain and a dysfunctional limb. Some ($n = 7$) also developed recurrent severe infections and wounds. No obvious clinical signs or history of nerve injury or other generalized neuropathy (i.e. polyneuropathy) were present at the time of diagnosis. Nerve conduction studies could not be carried out because of the excessive pain in the limbs. Limbs were dysfunctional for more than 1 year before amputation. All patients received many different (combinations of) treatments before their request for amputation including: exercise therapy, occupational therapy, manipulation, and partial immobilization (by means of splints); medication including morphine, anticonvulsants, antianxiety agents, and antidepressants; and electrotherapy, transcutaneous electrical nerve stimulation, and epidural spinal electrostimulation (Krans-Schreuder *et al.*, 2012). All treatments had yielded unsatisfactory results. Six patients underwent a surgical sympathectomy, which did not result in pain reduction.

Patients were seen by a team of specialists in this field (physiatrist, vascular surgeon, physiotherapist, and psychologist or psychiatrist) (Bodde *et al.*, 2014). Patients were seen by a psychiatrist or a psychologist to rule out severe psychiatric conditions such as body dysmorphic disorder.

After the decision to amputate was made, the patients were asked for permission to carry out a histopathological analysis of the amputated limb. After written consent was provided, the medical history was retrieved from the medical records. The following data were collected: age at amputation, sex, duration of CRPS-I before amputation, affected limb, level of amputation, and whether or not a sympathectomy was performed. The duration of CRPS-I was calculated from the time the patients fulfilled diagnostic IASP criteria in their medical record for the first time. The medical ethical committee was not involved. This scientific research does not involve treatment or intervention and therefore it is not bound to the law on scientific research on humans (Dutch = Wet Medisch Wetenschappelijk Onderzoek met mensen).

Tissue collection and sampling

Results from previous studies indicated damage of motor nerve fibers (Hulsman *et al.*, 2009). It was therefore logical to examine mixed, sensory, and motor nerves: ulnar, median, proximal radial, tibial, and peroneal nerves, and evaluate the loss of myelinated nerve fibers. However, as CRPS-I is a pain syndrome with autonomic dysregulation, we also aimed to evaluate nerves without motor fibers, namely, the sural and distal radial nerves. Moreover, for normal sural nerves, good morphometrical data exist for comparison with our results (O'Sullivan and Swallow, 1968).

Tissue samples from three patients were excluded: one patient had diabetes, which in itself can cause peripheral nerve pathology (e.g. decrease of fiber density) (Thomsen *et al.*, 2009); two patients were excluded because of limited quality of tissue processing/osmium-tetroxide (OsO_4) postfixation/impregnation.

Ultimately, tissue samples from 15 patients, three men and 12 women, median age 41 years [interquartile range (IQR): 35–47], were included. Four patients had undergone an amputation of an upper limb and 11 patients of a lower limb. The median duration of CRPS-I was 4 years (IQR: 2–9) (Table 1). Nerve biopsies of 4 cm were taken directly after amputation. Biopsies of the ulnar, median, and radial nerve were taken at the wrist level. In case of more proximal amputation, biopsies were also taken at the level of the elbow. Biopsies of the tibial and peroneal nerve were taken at the transtibial level and from the sural nerve just above the lateral malleolus. No other biopsies were taken in case of more proximal amputation of the lower limb. Biopsies were fixed in 2% glutaraldehyde with buffer for 1 week. Care was taken not to touch the nerve biopsies in the middle to prevent mechanical damage or artifacts. After the fixation period, a 1 mm thick section from the middle of the biopsy was taken, again to exclude material that might have been damaged during sampling (mostly found at the edges of a biopsy specimen) (Dyck *et al.*, 1982). This 1 mm thick tissue specimen was postfixed in OsO_4 for 2 days, embedded in Epon resin, and finally cut into semithin sections with a glass knife on a microtome at a thickness of 0.5 μm. The sections were mounted on glass slides and stained with toluidin blue for evaluation of nerve fiber loss and regeneration as well as morphometry.

Healthy sural nerve control group

The literature on morphometric analysis of human nerve fibers (nerve fiber diameters, density, and distribution) from healthy individuals is scarce. A list of the most common cited literature and its details is presented in Table 2 (O'Sullivan and Swallow, 1968; Ochoa and Mair, 1969; Jacobs and Love, 1985; Schröder *et al.*, 1988; Behse, 1990; Lindemuth *et al.*, 2002; Chentanez *et al.*, 2010). Most biopsies of the current study were from sural nerves ($n = 8$). We compared our results with results from a

Table 1 Characteristics of patients who underwent amputation because of CRPS-I and site of tissue sampling

Patient	Age at amputation (years)	Sex	Duration CRPS-I before amputation (years)	Affected limb	Level of amputation	Sympathectomy performed	Number of tissue samples	Tissue sample site
1	47	F	9	LL	KDA	−	3	tn (2), sn
2	45	F	7	UL	THA	+	6	rne, rnw, mne, mnw, une, unw
3	41	F	2	LL	TTA	−	3	pn (2), sn
4	16	F	4	LL	KDA	+	1	pn
5	25	F	2	LL	TFA	−	3	pn (2), sn
6	52	F	2	LL	TTA	−	5	pn (3), tn, sn
7	48	F	13	LL	TFA	−	1	tn
8	38	F	2	LL	KDA	+	2	pn, sn
9	35	F	5	LL	TFA	+	3	pn, tn, sn
10	23	M	1	UL	AF	−	3	rnw, mnw, unw
11	42	F	7	LL	TTA	+	2	pn, sn
12	49	M	3	LL	KDA	−	3	pn, tn, sn
13	36	F	13	LL	KDA	−	2	pn, tn
14	39	M	3	UL	AF	−	1	unw
15	44	F	20	UL	THA	+	5	rne, mne, mnw, une, unw

If there is more than one sample, the number of samples is provided within brackets.
−, no; +, yes; AF, amputation of forearm; F, female; KDA, knee-disarticulation; LL, lower limb; M, male; mne, median nerve elbow; mnw, median nerve wrist; pn, peroneal nerve; rne, radial nerve elbow; rnw, radial nerve wrist; sn, sural nerve; TFA, transfemoral amputation; THA, through humerus amputation; tn, tibial nerve; TTA, transtibial amputation; UL, upper limb; une, ulnar nerve elbow; unw, ulnar nerve wrist.

sample of sural nerves from individuals of approximately the same age as our patients from the study of O'Sullivan and Swallow (1968) [first seven patients in Appendix II from that study: median age 41 years (O'Sullivan and Swallow, 1968)].

In addition, we obtained healthy sural nerve biopsies from three patients undergoing cross face nerve grafts for a facial nerve paralysis, median age 32 years. Patients in this control group did not have peripheral nerve problems other than their facial nerve paralysis. The same method of preparation was used for these healthy sural biopsies. These biopsies were evaluated to verify whether our results in CRPS-I patients might be the result of our research and laboratory methodology.

Microscopic and morphometric analysis

All biopsies (Table 2) were assessed by M.B. and W.d.D. together using microscopy and morphometry. The biopsies were assessed for histological changes in the peripheral nerves (Fig. 1). Nerve injury causes Wallerian degeneration and fiber loss distally from the injury site, followed by fibrosis and possible nerve fiber regeneration. Therefore, the tissue characteristics assessed were fiber loss and degeneration (Fig. 1b) and regeneration (Fig. 1a,c and d). Fiber loss and degeneration were determined to be present if collapsed fibers or myelin balls were found. Regeneration was determined to be present if clusters of small myelinated fibers or normal-size axons with a thin myelin sheath were found.

For morphometric analysis, a magnification of ×400 was used to count all myelinated fibers within one standard area (9536.9 μm^2). Several standard areas per nerve were evaluated and the results were averaged. The areas were chosen randomly. To analyze fiber loss and regeneration in morphometry, all fibers with a myelin sheath per standard area were measured twice. First, the surface area of the total fiber was measured. Second, the axon surface area of the myelinated nerve fibers was measured. The fiber and axon diameter were calculated assuming circularity. Then, the *g*-ratio was calculated as the ratio between axon diameter and the total fiber diameter (Rushton, 1951; Friede and Beuche, 1985). The *g*-ratio represents the myelinization of an axon and it is a measure of maturity of the regenerating nerve. Regenerating fibers have thinner myelin sheaths, resulting in higher *g*-ratios (Schröder, 1972; Beuche and Friede, 1985; Friede and Beuche, 1985; King, 1999). We hypothesized a slightly higher *g*-ratio in our CRPS-I group because of regeneration of a relatively small number of fibers (Hulsman *et al.*, 2009).

Statistics/analytical approach

Descriptive data analysis was carried out using PASW for Windows, version 18.0 (SPSS, Chicago, USA). Fiber density and *g*-ratios were presented as means with SD and parametric tests were applied.

Microscopical variables (fiber loss and regeneration), axon, and fiber diameters were presented as median values with IQR and nonparametric tests (Mann–Whitney *U*-test) were applied.

Results

Nerve pathology in CRPS-I

Forty-three biopsies of nerve tissue were analyzed microscopically. In 35 biopsies (81%), fiber loss was found; in 37 biopsies (86%), regeneration was found. In three biopsies (7%), no signs of nerve pathology were found (samples taken from the median nerve at the elbow and radial nerve at the wrist in patient 2 and one sample from the peroneal nerve in patient 3; Table 1);

Table 2 Overview of morphometric data of peripheral nerves without evidence of peripheral nerve disease from the literature and data from the current study on peripheral nerves from patients with CRPS-I and healthy[a] controls

References	Participant characteristics [number of participants; age (years); mean and range]	Fiber diameter range	Nerve					
			Sural	Tibial	Peroneal	Radial	Median	Ulnar
O'Sullivan and Swallow (1968)		2–16 Peaks at 3–6 and 9–13 Proportion of large diameter fibers decreased with an increasing age in sural but not in radial nerves						
	8; 43 (26–57)		6015[c] (5340–6760) 7.50 ± 0.38					
	7; 41 (26–54)[b]		6050[c] (5340–6760) 7.40 ± 0.25					
	8; 40 (17–57)					7120[c] (5410–10 020) 7.42 ± 0.81		
Ochoa and Mair (1969)	6; 22.5 (15–34)	2–14 Peaks at 4–5 and 11	8000[d] (7000–10 000) – (Dyck *et al.* (1982); Jacobs and Love (1985)O'Sullivan and Swallow (1968)					
Dyck *et al.* (1982)	6; 32.5 (20–54)	1–13 Peaks at 3–4 and 10	8100[c] (7300–10 000) –					
	2; 26 and 42	1–14 Peak at 4 and 10		– (9300–10 200) –				
	3; 20, 26 and 42	1–15 Peaks at 4 and 9			10 500–12 000 –			
Jacobs and Love (1985)	6; 40 (21–58)	Peaks at 3–5 and 9–12	8065[c] (7760–10 190) –					
Schröder *et al.* (1988)	2; 16 and 17	– –	– –					– –
			6.24; 6.68					6.47; 8.77
Behse (1990)	9; 33 (17–54)	2–14 –	7700[c] (5500–8000), 6.74 ± 0.30					
Lindemuth *et al.* (2002)	6; 22–40	1–16 –	8397[d] ± 1583 5.9 ± 0.4	8345[d] ± 1118 5.9 ± 0.3				
Chentanez *et al.* (2010)	21; 36.6 (14–58)	1–16 –			8873[d] ± 167.4 6.32 ± 0.09			

This study	3[a]; 32	2–17	–	6062[c] (3989–9594) 8.08 ± 3.4						
	CRPS-I patients									
	8; 41.5 (25–52)	2–17	–		7078[c] (4299–8703) 6.78 ± 2.8					
	6; 47.5 (35–52)	2–17	–			5453[c] (1887–8388) 7.38 ± 3.4				
	9; 38 (16–52)	2–21	–				7025[c] (2306–8388) 7.03 ± 3.6			
	3; 23, 44 and 45	2–17	–					4823[c] (4194–7025) 8.49 ± 3.3		
	3; 23, 44 and 45	2–17	–						6920[c] (5662–8284) 8.12 ± 3.3	
	4; 23, 39, 44 and 45	2–17	–							5400[c] (3670–8179) 8.31 ± 3.4

All manuscripts included patients without evidence of peripheral nerve disease, except the current study. The authors derived the data from the appendices, tables, or text of the manuscripts cited. If information could not be derived, this is marked with: –. Regarding 'nerve data cells': in each cell, the first row represents the mean or the median fiber density: fibers/mm^2; the second row indicates the range, between brackets or SD; the third row represents the mean fiber diameter and SD. Fiber diameters, fiber diameter range, and peaks (in case of bimodal fiber diameter distribution) are expressed in μm.
CRPS-I, complex regional pain syndrome type I.
[a]Facial nerve paralysis, but otherwise healthy controls.
[b]Data from these patients were used for comparison in the current manuscript.
[c]Median fiber density.
[d]Mean fiber density.

however, in other nerve samples from these patients, signs of pathology were present. Therefore, in all patients, some pathological changes were found during microscopic analysis (nerve fiber loss or regeneration). In sural nerve biopsies from our control group, no signs of nerve fiber loss or regeneration were found.

Fiber density in CRPS-I and controls

A mean (SD) of 58.4 (16.9) myelinated fibers per standard area was found in CRPS-I tissue, corresponding with 6128 fibers/mm^2. Fiber densities of all CRPS-I and control nerves are presented in Table 2. Fiber densities varied considerably: the highest fiber density was found in a sural nerve sample of patient 6 (8703 fibers/mm^2) and the lowest fiber density was found in a tibial nerve sample of patient 1 (1887 fibers/mm^2), who also showed the most severe degenerative changes in the microscopical analysis.

Fiber diameters and distribution in CRPS-I and controls

Myelinated fiber diameters ranged from 2 to 21 μm. The median axon diameter of myelinated fibers was 3.3 μm (IQR: 2.3–4.7 μm) and the median fiber diameter of myelinated fibers was 7.1 μm (IQR: 4.3–10.3 μm). No significant differences in fiber size were found between biopsies taken at the elbow or at the wrist; therefore, biopsies taken from these two levels in the ulnar and median nerve were taken together. The median fiber diameters of CRPS-I and control nerves are presented in Table 2; line diagrams of fiber distribution of all nerves are presented in Figs 2 and 3. The percentages of fiber diameters greater than 12 μm are as follows: 33.7% radial nerve, 21.6% ulnar nerve, 15.9% median nerve, 15.4% tibial nerve, 11.6% peroneal nerve, and 2.4% sural nerves. In our healthy controls, this percentage was 18.9% (sural nerves).

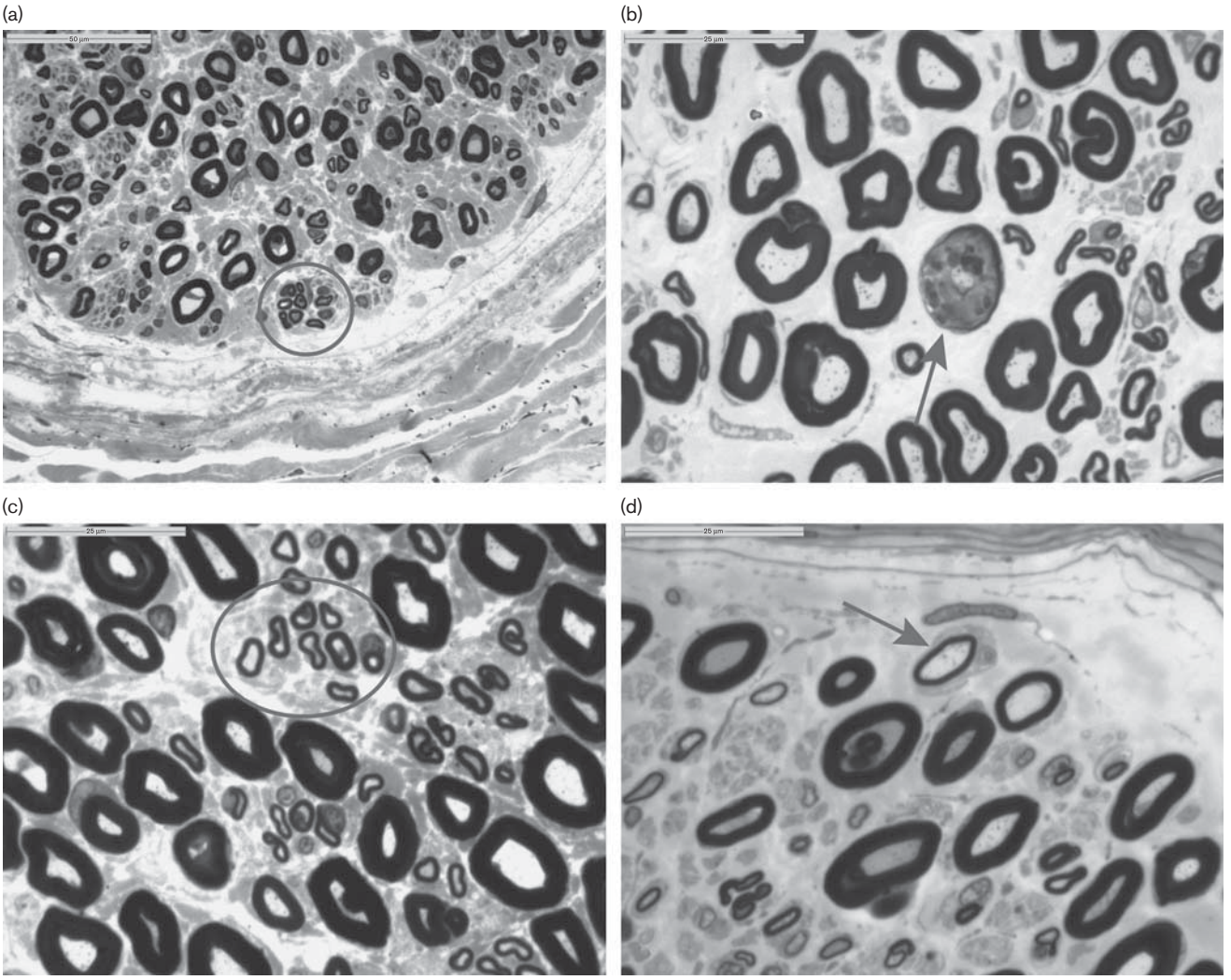
g-ratios

The mean (SD) *g*-ratio of all nerve fibers of CRPS-I patients was 0.50 (0.1); the mean *g*-ratio of the radial nerve biopsies was 0.53 (0.1). The *g*-ratio of the healthy controls [mean; 0.49, SD; 0.1 ($n = 724$ fibers)] was significantly ($P = 0.02$) different from the *g*-ratio of the sural nerves of CRPS-I patients [mean; 0.50, SD: 0.1 ($n = 500$ fibers)]. This difference indicates slightly thinner myelin sheaths in the CRPS-I group, in turn pointing to the direction of nerve fiber regeneration.

Influence of sympathectomy

The samples of patients with ($n = 24$) and without ($n = 19$) sympathectomy were compared (Fig. 2b). The results were in agreement with what could be expected as in sympathectomy smaller nerve fibers are affected and a larger percentage of large nerve fibers will remain.

Fig. 1



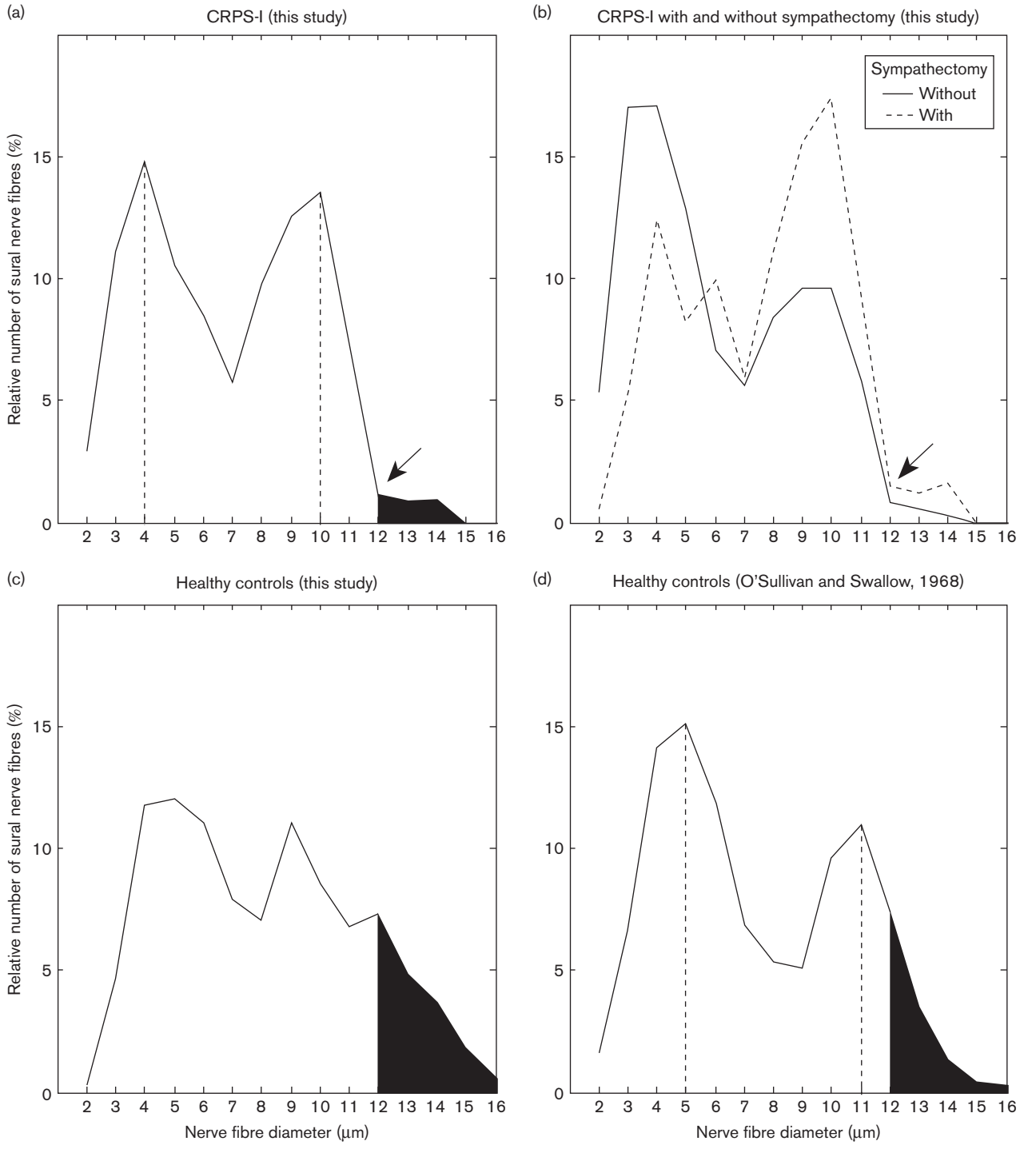
(a) Overview of a peripheral nerve of a CRPS-1 patient (bar represents 50 μm). The circle indicates a small regeneration cluster. In (b), a degenerating nerve fiber is shown (arrow). (c) Regeneration cluster (circle). The arrow in (d) indicates an regenerated nerve fiber with a relatively thin myelin sheath. Bars in (b–d): 25 μm. CRPS-1, complex regional pain syndrome type I.

Discussion

CRPS-I is defined as a syndrome without nerve injury (Stanton-Hicks *et al.*, 1995). However, our results indicate that peripheral nerve damage is present in most of our CRPS-1 nerve tissue. Whether nerve damage triggers the syndrome or occurs during the course of the syndrome remains unknown from this study. These results are in agreement with the signs of a process of repetitive denervation and reinnervation of muscle tissue found previously and this has been suggested by others (Van der Laan *et al.*, 1998a; Albrecht *et al.*, 2006; Oaklander *et al.*, 2006; Hulsman *et al.*, 2009).

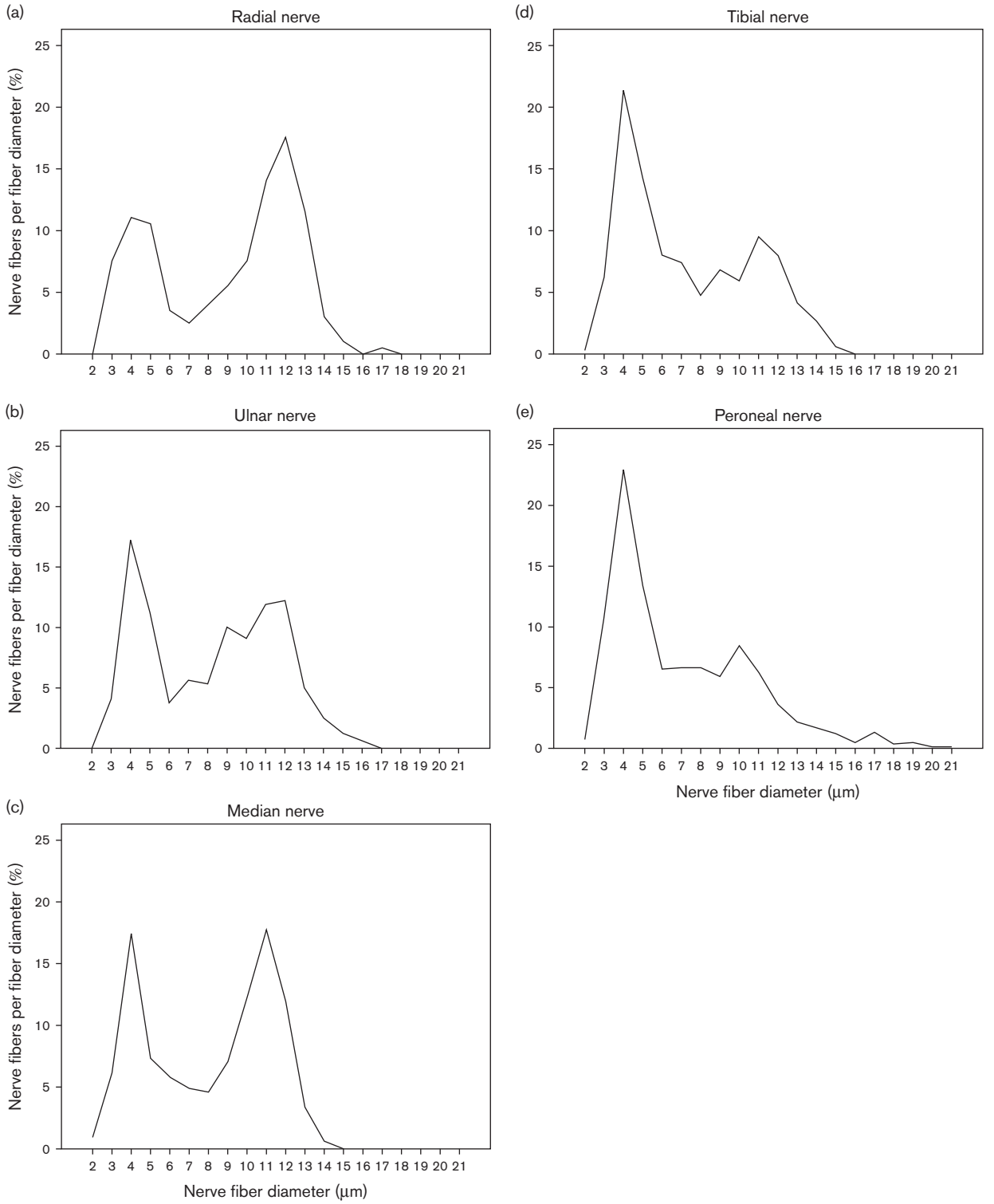
All CRPS-I patients showed, to a varying extent, histological changes including nerve fiber loss and regeneration. These histological changes were also found by Van der Laan *et al.* (1998a). These histological abnormalities were not observed in the sural nerve biopsies from our healthy controls, although it has been described that some structural abnormalities may be found in healthy controls (Behse, 1990; Dyck *et al.*, 2005). Our microscopic findings suggest that peripheral nerve degeneration and regeneration does occur during the course of CRPS-I, at least in those who had their limb amputated. It has been suggested that CRPS-I is a syndrome in which distally located peripheral nerve fibers (for instance the nerve endings in the skin) are damaged (Oaklander *et al.*, 2006; Oaklander and Fields, 2009). Our findings indicate that nerve damage is also present more proximally in the nerves. Several hypotheses for this damage at a more proximal level can be formulated. The nerve may be damaged through local pressure from edema in the

Fig. 2



(a) Diameter distribution of myelinated nerve fibers from sural nerve biopsies in our patient group (eight patients, median age 41.5 years) in percentages. Arrow: decrease in large fibers is visible 12 μm or more. The black area under the curve shows the amount of large fibers present. (b) Diameter distribution of myelinated nerve fibers from sural nerve biopsies in our patient group comparing patients with and without sympathectomy. Patients with a sympathectomy have relatively smaller amounts of small myelinated nerve fibers and larger myelinated nerve fibers. Arrow: in both groups, a decrease in larger fibers is present from about 12 μm. (c) Diameter distribution of myelinated nerve fibers from sural nerve biopsies from our healthy control group (nerves biopsies taken from patients undergoing a cross facial nerve graft because of facial nerve paralysis). The black area under the curve shows the amount of large fibers (≥12 μm) present. (d) Distribution of diameter of myelinated nerve fibers from sural nerve biopsies from the literature (O'Sullivan and Swallow, 1968: seven patients, median age 41 years). The black area under the curve shows the amount of large fibers (≥12 μm) present. A larger area under the curve is seen in (d) compared with (a). Nerve fiber diameters in sural nerves in the literature show a bimodal distribution (d). Distribution of nerve fiber diameters in our small healthy sural nerve control group showed fewer clear peaks (c); however, the bimodal distribution is present in the CRPS-I group (a). CRPS-1, complex regional pain syndrome type I.

Fig. 3



Diameter distribution of nerve fibers of the radial nerve (a), ulnar nerve (b), median nerve (c), tibial nerve (d), and peroneal nerve (e) from patients who had undergone amputation because of CRPS-I. CRPS-1, complex regional pain syndrome type I.

affected limb. An animal model of rats with chronic loose ligatures of the sciatic nerve shows a deficit in large myelinated nerve fibers (>9 μm) at the end of the observation period at 10 weeks when swelling had disappeared and the total number of myelinated fibers was close to normal (Guilbaud *et al.*, 1993). Nerves may also be damaged through other local symptoms of autonomic dysfunction and trophic changes. These changes can lead to endothelial dysfunction and the production of free radicals, which in turn induce histopathological changes caused by oxidative stress (Coderre and Bennett, 2008). Yet another hypothesis in the pathophysiology of CRPS-I is the concept of neurogenic inflammation in which local or systemic 'products' such as neuropeptides and cytokines may cause (local) damage of the nervous system (Birklein *et al.*, 2001; Weber *et al.*, 2001; Marinus *et al.*, 2011). Finally, nerve degeneration and regeneration may also be the result of retrograde degeneration of the nerve after damage more distally in an extremity.

Morphometric characteristics of peripheral nerve fibers were independent of age in a group ranging in age from 14 to 58 years (mean 36.6) (Chentanez *et al.*, 2010). Fiber density remains relatively constant until the age of 60 years. Thereafter, it tends to decrease (Jacobs and Love, 1985). Therefore, the results from these studies can be used for comparison with our data. Fiber densities reported in the literature range considerably from ~5300 to 12000/mm^2 depending on the nerve in question (Table 2). The fiber density in the sural nerves of our control group matched those from O'Sullivan and Swallow (1968), but was smaller when compared with Jacobs and Love (1985), Behse (1990), and Dyck *et al.* (2005) (Table 2). Because of the wide range of sural nerve control data in the literature, our CRPS-I data should be evaluated with caution. In our CRPS-I patient group, the fiber density also varied considerably and was within the range of our control group. Therefore, hard conclusions cannot be drawn from these data comparisons. Most likely, this means that even though nerve fibers pathology can be found in all our patients, the number of nerve fibers that are affected is too small to cause significant changes in nerve fiber density measurements. Besides the sural nerve, control data for the other nerves from literature are even scarcer (Table 2) and therefore conclusions cannot be drawn.

Completed maturation of axon and myelin sheath is considered to occur around the age of 17 years (Schröder *et al.*, 1988). Nerve fiber diameter ranges from 2 to ~21 μm (King, 1999). Sural nerves are known to have a bimodal fiber diameter distribution with peaks at 3–5 μm and at 9–12 μm at adult age (Fig. 2d) (Jacobs and Love, 1985). However, others found a decrease in the proportion of large diameter fibers with increasing age in the sural nerve, but not the radial nerve (O'Sullivan and Swallow, 1968). To make adequate comparisons of our data with data from the literature, we used data from sural nerves from patients of a similar age range. Some analysis methods allow counting nerve fibers less than 1 μm (Dyck *et al.*, 1982; Lindemuth *et al.*, 2002; Chentanez *et al.*, 2010), resulting in smaller mean fiber diameters (Table 2) and making adequate comparisons difficult.

The distribution of nerve fiber diameters from the sural nerve of our control group showed less clear peaks (Fig. 2c); however, the bimodal distribution is present in the CRPS-I group (Fig. 2a). Interestingly, the area under the curve at greater than 12 μm shows a loss of large myelinated nerve fibers in the CRPS-I group (Fig. 2a). This loss of larger myelinated nerve fibers cannot be explained by artifacts because of surgical removal or different fixation and measuring techniques because the same methods were applied in our control sural nerves. Theoretically, a sympathectomy would cause a combination of reduced numbers of unmyelinated and thin myelinated nerve fibers because of Wallerian degeneration and this will influence the mean nerve fiber diameter. The decrease at 12 μm existed in both the CRPS-I group with and without sympathectomy. This means that this form of therapeutic intervention does not cause the decrease of nerve fibers greater than 12 μm.

On the basis of the findings in the sural nerves described here, as well as the previously reported skeletal muscle pathology, we expected a similar loss of large myelinated fibers in the mixed sensory-motor nerves. However, the percentages of fiber diameters greater than 12 μm in the other nerves were much higher. Therefore, it seems that our findings from the CRPS-I sural nerves cannot be translated into the other CRPS-I nerves.

A limitation of this study is the small sample of control sural nerves and the lack of control samples from mixed (motor) nerve fibers. As stated, data from the literature are difficult to compare when samples are not taken and measured following an identical procedure (O'Sullivan and Swallow, 1968). Morphometric data of healthy nerves are scarce and often not extensive enough for good comparison (Table 2). Limbs are generally amputated because of vascular problems, which may cause impaired and changed vascularization of peripheral nerves, or because of oncological reasons. In those cases, changes in nerve tissue may have occurred because of systemic or local chemotherapy. Thus, the above-mentioned limbs are not suitable to serve as 'healthy' control material. In the Netherlands, bodies available for anatomical research are anonymous. Consequently, the medical backgrounds are unknown and we cannot reliably use them as healthy controls. This is a common problem in morphometric research, although others may choose to use postmortem samples (Thomsen *et al.*, 2009).

Future research

As the sural nerve samples showed more marked changes in the morphometrical evaluation than the other nerves

studied here, fiber typing by immunohistochemistry could be interesting. Furthermore, the evaluation of the motor nerve fibers and end-plates in the skeletal muscle tissue could provide additional information on the pathophysiology of the muscular changes in CRPS-I.

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Conflicts of interest

There are no conflicts of interest.

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