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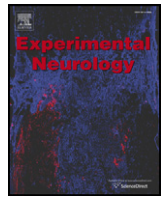
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## Cutaneous noradrenaline measured by microdialysis in complex regional pain syndrome during whole-body cooling and heating

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## ABSTRACT

Complex regional pain syndrome (CRPS) is characterised by autonomic, sensory, and motor disturbances. The underlying mechanisms of the autonomic changes in CRPS are unknown. However, it has been postulated that sympathetic inhibition in the acute phase with locally reduced levels of noradrenaline is followed by an up-regulation of alpha-adrenoceptors in chronic CRPS leading to denervation supersensitivity to catecholamines. This exploratory study examined the effect of cutaneous sympathetic activation and inhibition on cutaneous noradrenaline release, vascular reactivity, and pain in CRPS patients and in healthy volunteers. Seven patients and nine controls completed whole-body cooling (sympathetic activation) and heating (sympathetic inhibition) induced by a whole-body thermal suit with simultaneous measurement of the skin temperature, skin blood flow, and release of dermal noradrenaline. CRPS pain and the perceived skin temperature were measured every 5 min during thermal exposure, while noradrenaline was determined from cutaneous microdialysate collected every 20 min throughout the study period. Cooling induced peripheral sympathetic activation in patients and controls with significant increases in dermal noradrenaline, vasoconstriction, and reduction in skin temperature. The main findings were that the noradrenaline response did not differ between patients and controls or between the CRPS hand and the contralateral unaffected hand, suggesting that the evoked noradrenaline release from the cutaneous sympathetic postganglionic fibres is preserved in chronic CRPS patients.

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## Introduction

Complex regional pain syndrome (CRPS) is a heterogeneous pain condition characterised by pain, autonomic disturbances, trophic changes, and reduced motor function in the affected limb (Veldman et al., 1993). CRPS pain has been attributed to abnormal activity of the sympathetic nervous system due to clinical features with disturbances in autonomic functions such as the regulation of skin temperature, colour, blood flow,

and sweat secretion, as well as the occurrence of oedema (Veldman et al., 1993).

A number of findings have indicated that peripheral noradrenaline (NE) release plays a central role in CRPS. Firstly, intradermal injection of NE evokes pain in patients with sympathetically maintained pain (Ali et al., 2000). Secondly, spontaneous and evoked pain increases following sympathetic arousal (Drummond et al., 2001) and during physiological activation of cutaneous vasoconstrictor neurons projecting to the CRPS limb (Baron et al., 2002). Thirdly, lower plasma levels of NE (Drummond et al., 1991; Harden et al., 1994; Wasner et al., 1999) and its neuronal metabolite 3,4-dihydroxyphenylethyleneglycol (Drummond et al., 1991) have been detected in the painful limb compared with the contralateral limb in patients with CRPS. Finally, Arnold et al. (1993) reported an increased alpha-adrenoceptor responsiveness of the dorsal superficial hand veins in CRPS. Based on these findings, it has been suggested that partial sympathetic denervation in the acute phase with locally reduced levels of NE is followed by an up-regulation of alpha-adrenoceptors on sensory fibres (possibly on nociceptive fibres)

**Abbreviations:** CRPS, complex regional pain syndrome; NE, noradrenaline; NRS, numeric rating scale; LDF, laser Doppler flowmeter.

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in the chronic stage of CRPS (Drummond et al., 1991). Indeed a small autoradiographic study of five CRPS patients found a greater density of alpha1-adrenoceptors in the epidermis and upper dermis of patients' hyperalgesic skin compared to the skin of healthy controls (Drummond et al., 1996).

While these findings suggest that local NE plays a pathophysiological role in CRPS, the evidence for pain relief by sympathectomy in CRPS is limited. So far, there is no evidence from randomised, double-blinded, controlled trials on the efficacy and safety of chemical and surgical sympathectomy for neuropathic pain (Furlan et al., 2000; Straube et al., 2010). Similarly, the evidence for pain relief in CRPS by local anaesthetic sympathetic blocks is scarce (Cepeda et al., 2005).

Therefore, it is still unclear whether NE is changed locally at the site of injury and whether it plays a role in the maintenance of pain in CRPS. To determine the possible role of NE in the pain associated with CRPS, it is necessary to measure NE levels under controlled conditions in the vicinity of dermal nociception in areas with evoked pain. One way to achieve this is to "clamp" the peripheral sympathetic nervous system and measure the release of NE to the skin when the sympathetic nervous system is either activated or inhibited.

Whole-body cooling is a known physiological stimulus that effectively activates peripheral cutaneous sympathetic fibres resulting in the release of cutaneous NE (Bini et al., 1980a). Thus, in the present exploratory study, cutaneous NE was measured in the hyperalgesic zone by microdialysis during rest and during whole-body cooling and heating in patients with CRPS as well as in a gender- and age-matched group of healthy controls. The primary aim was to study NE regulation during cooling and heating in patients and to compare the NE levels to those of healthy controls. The secondary aim was to test for a correlation between changes in NE release and CRPS pain. The level of sympathetic activation was evaluated by various autonomic measures.

## Materials and methods

### Ethical approval

The study conformed to the standards set by the latest revision of the *Declaration of Helsinki* and was approved by the Local Ethics Committee (No. 20050192) and The Danish Data Protection Agency. The patients and healthy controls received written and oral information about the study and gave their written informed consent.

### Subjects

The inclusion criteria were: At least 18 years of age and a normal ECG. Participants did not receive pharmacological treatment potentially affecting the vascular or autonomic systems. Thus, no included patient was tested for sympathetically maintained pain prior to the experiment. Pregnant or lactating women were excluded, as were patients with cardiovascular or endocrine diseases (including diabetes), malignancies, acute or chronic infections (including known human immunodeficiency virus infection), known alcohol or drug abuse, or pain conditions other than the CRPS.

Ten CRPS patients attending the Neuropathic Pain Clinic at Aarhus University Hospital, Aarhus, Denmark were included. The patients had symptoms in one upper limb and fulfilled the 'Budapest' research diagnostic criteria for CRPS (Bruehl et al., 1999). Three patients did not complete the thermal exposure; one stopped after baseline measurements due to spontaneous pain aggravation before the thermal exposure, one stopped after baseline and cooling due to severe evoked pain, and one could not finish the cooling session due to cold hyperalgesia on the chest but completed the baseline and heating measurements. Thus, seven patients completed the thermal exposure.

Ten age- and gender-matched healthy volunteers were recruited by advertising at Aarhus University Hospital. The inclusion criteria were a

normal medical history and physical examination. One healthy control did not complete the cooling and heating sessions due to discomfort.

### Medical pain history and clinical examination

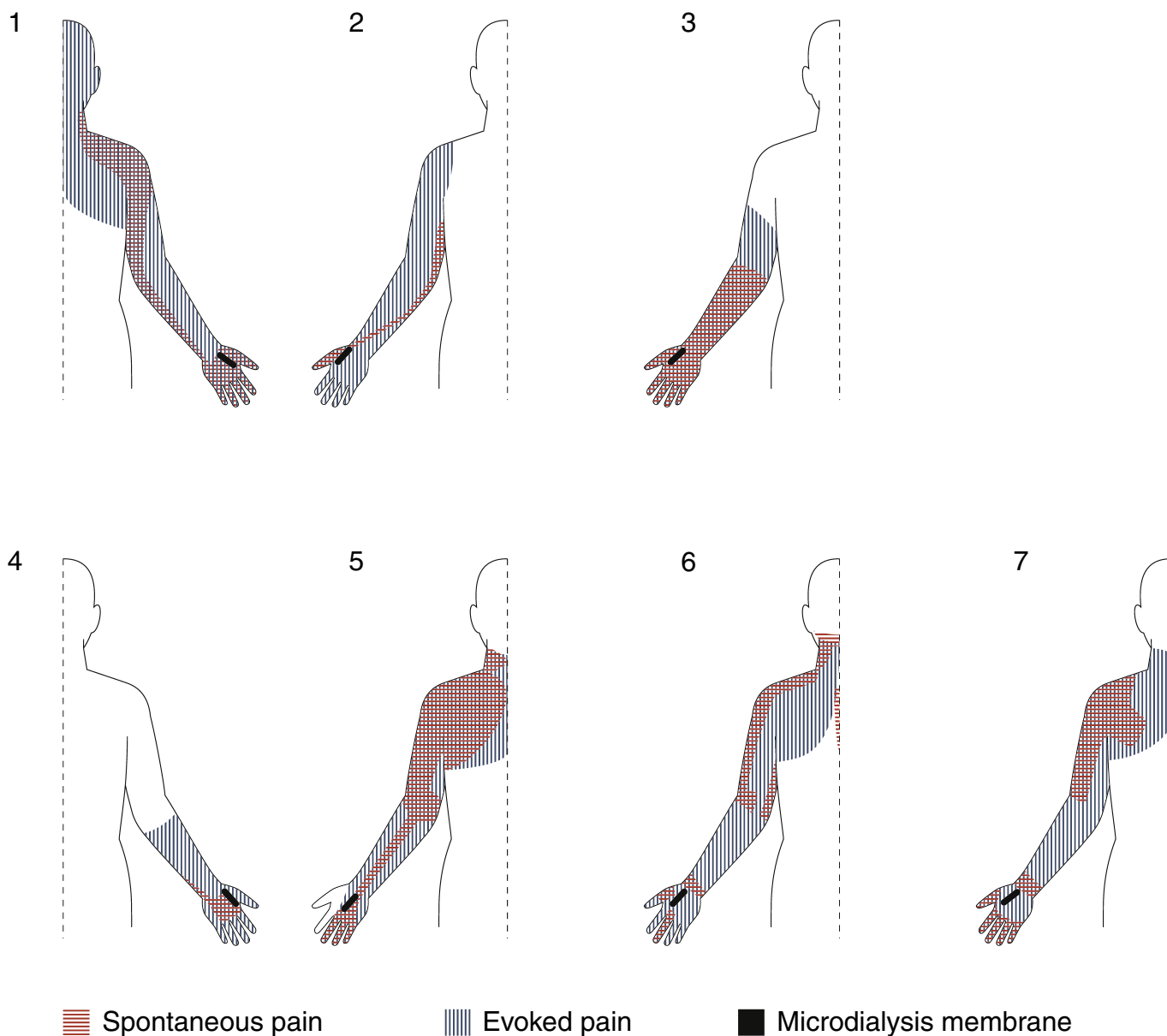
The medical history was obtained, and the patients rated their spontaneous limb pain, the average limb pain within the last 24 h, and the highest limb pain intensity during the last 24 h on a numeric rating scale (NRS) from 0 to 10 (0 = no pain, 10 = maximal imaginable pain). Each patient marked the area of spontaneous pain on a body chart. The neurological examination was supplemented with a sensory examination that mapped the areas of pinprick hyperalgesia and brush allodynia. Pinprick hyperalgesia was defined as increased pain induced by a von Frey monofilament (745 mN, Semmes-Weinstein, Stoelting, IL) and assessed in distributional steps of 10 mm. Brush allodynia or dysesthesia was induced by brushing at 5 cm/s (SENSELab, Brush-05, Somedic Sales AB, Hörby, Sweden) in similar steps.

### Whole-body cooling and heating and ratings of subjective skin temperature during the thermal challenge

A thermal suit covering the whole body apart from the feet, forearms, head, and neck was used to induce whole-body cooling and heating (Baron et al., 2002). The forearms were not covered by the suit to avoid direct heating and cooling of the area of microdialysis. Circulating water of either 5 °C or 50 °C induced, respectively, cooling (sympathetic activation with high cutaneous vasoconstriction) and heating (sympathetic inhibition with low cutaneous vasoconstriction). During the thermal exposure the patients and controls every 5 min rated their perceived skin temperature on a scale from -4 to +4 (McAllen et al., 2006): +4 very hot, +3 hot, +2 warm, +1 slightly warm, 0 neutral, -1 slightly cool, -2 cool, -3 cold, and -4 very cold. The cooling and heating sessions each continued for at least 40 min until the skin temperature of the unaffected limb was below 26 °C and the participant gave a rating of -4 very cold or until the skin temperature was more than 34 °C and the participant gave a rating of +4 very hot, respectively. If an unacceptable level of pain or unpleasantness occurred before reaching 26 °C and a rating of -4 or before reaching 34 °C and a rating of +4 further cooling and heating was stopped. However, on all occasions participants finalized the ongoing 20 min session for the collection of the microdialysis perfusate.

### Dermal microdialysis

Sterile microdialysis probes with a membrane diameter/length of 0.5/30 mm and a molecular weight cut-off of 20 kDa (CMA 66, CMA Microdialysis AB, Solna, Sweden) were inserted intradermally in the dorsum of the hand between the first and second metacarpal bone of both hands carefully avoiding damage to the visible cutaneous vessels. In CRPS patients, the microdialysis probes were inserted at skin sites with spontaneous or evoked pain (Fig. 1). An ice pack was placed on the skin for approximately 1 min before the insertion of the probe to avoid haematoma and to induce slight analgesia. The skin was swabbed with ethanol 70% before intradermal insertion of the guide cannula (21 Gauge) to a length of 40 mm, ensuring that the dialysis membrane was covered by skin along its entire length. The probes were perfused with sterile Ringers solution at 20 µl/min for 5 min and then 2 µl/min using a microdialysis pump (CMA 402, CMA Microdialysis AB, Solna, Sweden). No samples were collected for the first hour after the insertion of the probes (the trauma phase). Subsequently, samples were collected every 20 min throughout the study period. Samples of 40 µl dialysate were collected into light-impermeable vials containing 4 µl of a solution with EGTA (Merck KGaA, Darmstadt, Germany) and reduced glutathione (Merck KGaA, Darmstadt, Germany). The vials were placed in ice water during the sampling of the dialysate and immediately frozen at -80 °C until analysis.



**Fig. 1.** Areas (posterior dimensions) of spontaneous and evoked pain (pinprick hyperalgesia and brush allodynia) in patients with complex regional pain syndrome (CRPS). The microdialysis probe was placed in an area with spontaneous or evoked pain.

To confirm the ability of the microdialysis technique to monitor human intradermal NE changes, pilot experiments (two probes, one subject) were performed with intra-probe delivery of tyramine (Leis et al., 2004) (VWR – Bie & Berntsen, Herlev, Denmark). Tyramine is an indirect sympathomimetic preventing NE reuptake and displacing NE from neuronal storage vesicles (Borgen and Iversen, 1965). Tyramine (10 µg/ml) infused for 20 min increased dermal levels of NE more than 10-fold over the baseline levels of NE (data not shown).

#### Analysis of NE

Concentrations of NE in the skin microdialysis samples were determined with HPLC with electrochemical detection based on a slight modification of the method as described elsewhere (Yoshitake et al., 2010). Briefly, the HPLC system consisted of a HTEC500 liquid chromatograph, including an electrochemical detector (Eicom, Kyoto, Japan), a CMA/200 Refrigerated Microsampler (CMA Microdialysis, Stockholm, Sweden), and a Clarity data acquisition system (DataApex, Prague, The Czech

Republic). The potential of the glassy carbon working electrode was set to +450 mV vs. the Ag/AgCl reference electrode. NE was separated on an Eicompak CAX (200×2.0 mm; Eicom) column using a mobile phase consisting of 10% methanol and 90% of 0.1 M phosphate buffer (pH 6.0) and containing 15 mM potassium chloride and 65 µM EDTA-2Na. The detection limit (signal-to-noise ratio=3) for NE was 0.05 nM, that is 0.75 fmol in 15 µl injected onto the column.

#### Cutaneous blood flow and temperature

Skin blood flow and temperature were measured continuously with two laser Doppler flowmeters (LDF) with combined optic/temperature probes placed bilaterally at the pulp of the first finger (MoorVMF, Moor Inc., Axminster, Devon, UK; 40 Hz) and bilaterally on the dorsum of the hands in the area of microdialysis (DRT4, Moor Inc., Axminster, Devon, UK; 1 Hz). The LDF probes were attached to the skin using double-adhesive tape and adequate probe holders. LDF arbitrary perfusion units (a.u.) and skin temperature (data not shown) were averaged for periods

of 20 min during baseline, the last 20 min of cooling, and the last 20 min of heating for further statistical analysis. The skin temperature was also measured manually every 5 min in digits two through five with an infrared thermometer (IR Thermometer, IR-1000 L, Voltcraft, Hirschau, Germany).

#### Core temperature

The core temperature was measured with an ear thermometer (ThermoScan 6013, Braun, Kronberg, Germany) at baseline, and after 5 min of the last 20 min of cooling and heating.

#### Ratings of pain during the thermal challenge

Every 5 min during the thermal challenge, the patients rated the pain in the affected arm on an 11-point NRS (0 = no pain, 10 = maximal imaginable pain).

#### Autonomic and hemodynamic parameters

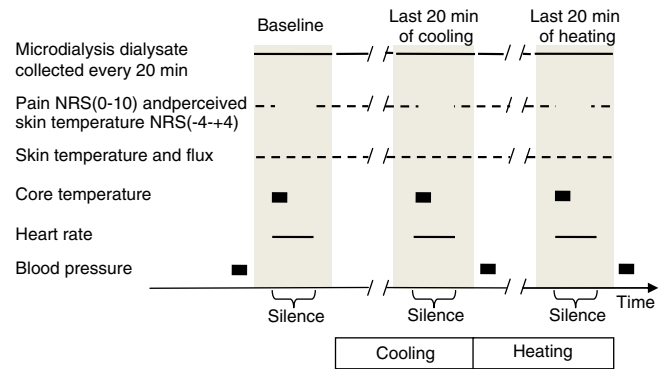
A Task Force Monitor (CNSystems Medizintechnik AG, Graz, Austria) non-invasively recorded the ECG (sample rate: 1000 Hz) and oscillometric blood pressure (Fortin et al., 2006). Hemodynamic parameters were then estimated and expressed as the mean values of RR intervals (mean time between consecutive normal R waves in the QRS complexes), and systolic and diastolic blood pressure.

#### Experimental setup

All subjects were investigated in the morning after fasting overnight or from 3 p.m. after at least 4 h of fasting. The subjects had abstained from physical activity for at least 24 h and caffeine-containing beverages, alcohol, and smoking for at least 12 h. Subjects emptied their bladder before the experiments. They were examined in the supine position in a room with a mean temperature of 24.2 °C (range 22.5–25.8 °C). The arms were immobilised in a semi-flexed position. Measurements of skin temperature and flux as well as reports of pain and perceived skin temperature were made during resting, cooling, and heating with simultaneous collection of the microdialysis dialysate. Heart rates were measured in silence during three 10-min periods at baseline, and during the last 20 min of cooling and heating (Fig. 2). Recordings were initiated 5 min after the start of the specific period.

#### Statistical analysis

Statistical tests and figures are based on the complete data set, which was available for seven patients. For healthy controls, the statistical tests and figures are based on the complete data set, which was available for seven controls for the NE, for eight controls for the blood pressure and flux, and for nine controls for the remaining measures. However, data from all ten patients and ten controls are included in Figs. 3C–F. The measurements are presented as the means and SD. Fisher's exact test was used to compare the dichotomous clinical variables, and unpaired t-tests for differences in quantitative clinical variables. The difference between the groups (patients and controls) with respect to core temperature, autonomic measurements, and NE levels during the three conditions (baseline, last 20 min of cooling and heating) was assessed using 2-way repeated measures ANOVA, as was the difference in NE levels between the hands in the CRPS patients. The post hoc Wilcoxon signed-rank test was used to assess the significant main effect of the condition (last 20 min of cooling versus last 20 min of heating), which was the primary outcome. Spearman's rho was used for correlation analysis. To test whether the NE response on the CRPS hand differed from the contralateral hand, in each patient, the differences in NE between the hands were calculated for each collection point and summarised as the average difference of all measurements



**Fig. 2.** Experimental set-up. Measurements were performed during baseline (20 min supine rest), cooling, and heating. Ten min of silence occurred during baseline, and during the last 20 min of cooling and heating during which neither experimenters nor volunteers spoke. The microdialysis dialysate was collected every 20 min throughout the experiment. Pain and subjectively perceived skin temperature were rated every 5 min on numeric rating scales (NRS), except during the silent periods. Skin temperature and flux were measured continuously. The core temperature was measured during baseline, and during the last 20 min of cooling and heating. Autonomic measurements obtained with the Task Force Monitor (TFM) were performed during the silent periods. Blood pressure was measured before baseline, after cooling, and after heating.

collected during each separate condition: baseline, cooling, and heating. The Wilcoxon signed rank test was used to assess if these average differences deviated significantly from zero. All statistical tests were two-sided, and the level of significance was 5%. Stata 8.1 (StataCorp. 2003, Stata Statistical Software: Release 8.0. College Station, TX: Stata Corporation) was used for the basic statistical calculations.

## Results

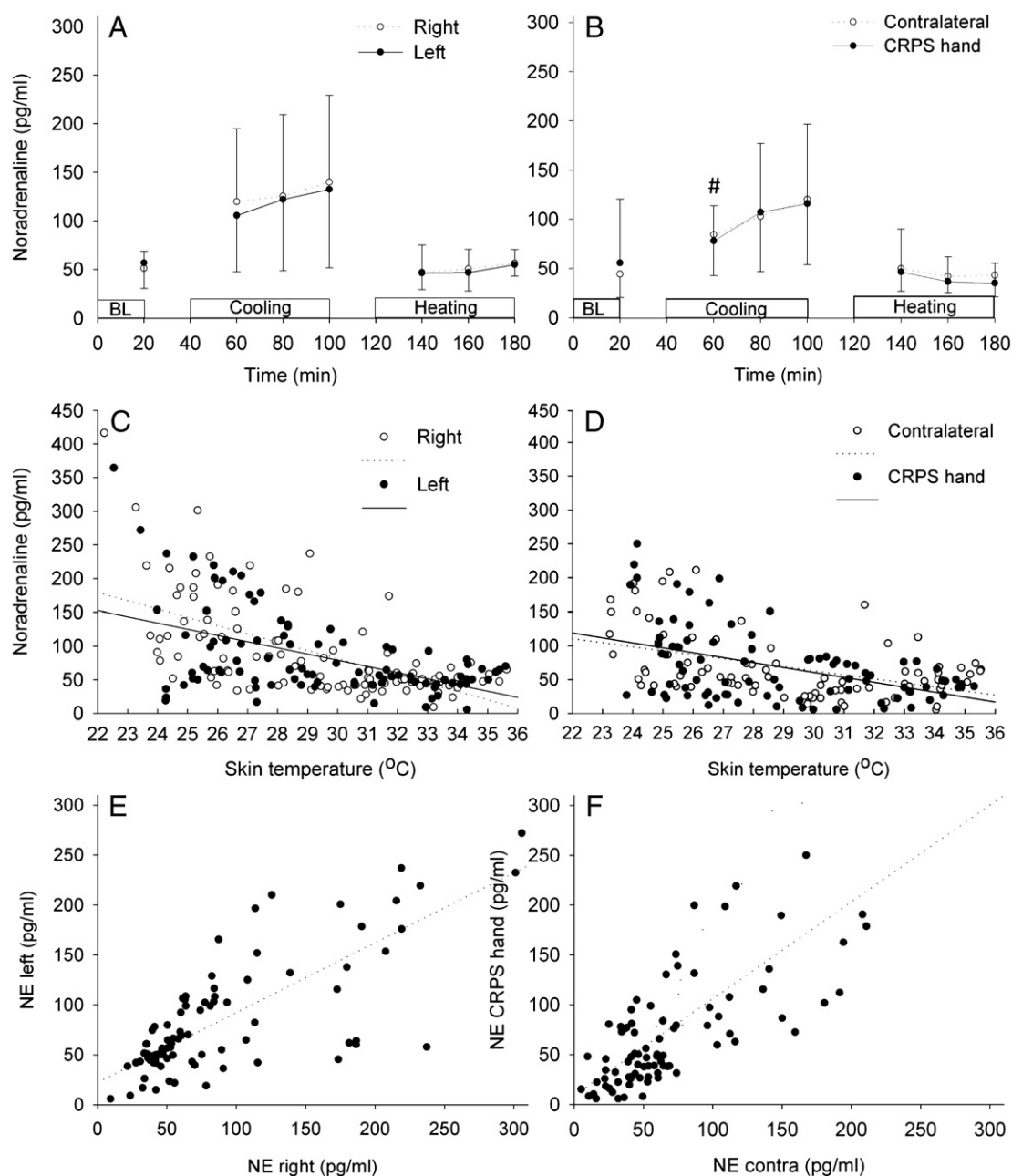
#### Patient characteristics

The patients (mean age 47 years, SD 12 years; female/male ratio: 5/2; body mass index 27 kg/m<sup>2</sup>, SD 4 kg/m<sup>2</sup>) and age- and gender-matched controls (mean age 47 years, SD 14 years; female/male ratio: 4/5; body mass index 26 kg/m<sup>2</sup>, SD 5 kg/m<sup>2</sup>) did not differ regarding weight, height, and body mass index. The number of smoking patients (57%) and controls (33%) did not differ ( $P = 0.62$ ).

The inciting events in the CRPS patients were the following: Carpal tunnel surgery, crush injury at the proximal phalanx of digit 1, tennis elbow surgery, ulnar nerve lesion, surgical decompression of the ulnar nerve, comminuted fracture of the proximal humerus, and wrist distortion. The patients were medicated as follows: In one patient, amitriptyline was gradually reduced and not taken for six days prior to study start. Four patients were taking acetaminophen, three tramadol, two pregabalin, and one subject was treated with buprenorphine plaster, one with darifenacin, one with alendronate, and one with glucosamine. The spontaneous pain, allodynia, and hyperalgesia in the CRPS patients were distally localised and not limited to the territory of a single peripheral nerve (Fig. 1). The mean CRPS limb pain at the study visit was 6.2 (range 5–8). It was 6.4 (range 4.5–8) over the previous 24 h, and the maximum pain over the previous 24 h was 8.5 (range 6–10). The mean pain duration was 1795 (range 147–4363) days. Thus all patients had chronic CRPS according to the most recently established criteria for CRPS (Bruehl et al., 1999).

#### Cutaneous NE

For NE (Table 1, Figs. 3A and B), there was a significant condition effect with an increase in NE during high versus low peripheral sympathetic activation by a factor of 3.4 on the CRPS hand and the contralateral pain-free hand. In controls, the mean NE for both hands increased significantly by a factor of 2.3. There was no difference



**Fig. 3.** Cutaneous noradrenaline (NE) levels during whole-body cooling and heating. There was a significant release of NE during peripheral sympathetic activation (cooling) compared to peripheral sympathetic inhibition (heating) in controls (A) and patients (B) (mean (SD)). There was no difference between the hands in the patients or the controls. For each group the responses in the hands were similar during baseline, cooling, and heating. # One patient was only cooled for 40 min and is not included in the time period from 40 to 60 min. The data for skin temperature versus NE concentration in all included controls (C) and patients (D) show increased NE release at the lowered skin temperature. For all included controls, NE samples collected during baseline, cooling, and heating from the right hand are plotted against the values from the left hand (E). For all included patients, the NE samples collected during baseline, cooling, and heating from the affected hand (NE CRPS hand) are plotted against the values from the unaffected contralateral hand (NE contra) (F).

between the hands in the patients or the controls. For each group, the responses in the hands were similar during baseline, cooling, and heating. Comparing patients to controls (mean NE of both hands), the responses were similar during baseline, cooling, and heating.

For patients and controls, NE levels increased when the skin temperature was lowered (Figs. 3C and D). During baseline there was no correlation between mean skin temperatures and mean NE levels in the controls ( $r = -0.32$ ,  $P = 0.21$ ) or in the patients ( $r = -0.22$ ,  $P = 0.35$ ).

The NE release on the CRPS hand was comparable to the contralateral hand (Fig. 3E;  $P = 0.80$ ). The NE release was not significantly different in the two hands in the controls (Fig. 3F;  $P = 0.86$ ).

#### Modulation by whole-body cooling and heating

The mean duration of cooling in the patients was 89 min (SD 53, range 40–200), and in the controls, it was 109 min (SD 32, range 80–160;  $P = 0.35$ ). The mean length of the heating session among the patients was 97 min (SD 21, range 60–120) versus 100 min (SD 26, range 60–140) for the controls ( $P = 0.82$ ). Cooling and heating did not affect the core temperature. Regarding 1) the skin temperature measured at pulps of digits two to five (Figs. 4A and B, Table 1), 2) flux measured at digit one (Figs. 4C and D, Table 1), and 3) flux measured near the microdialysis probe (Figs. 4E and F, Table 1) there was a significant condition

**Table 1**  
Cutaneous noradrenaline, skin temperature, and flux in patients and controls exposed to cooling and heating.

	Baseline	Cold	Heat	ANOVA (P value)			Wilcoxon
				Hand × condition	Between-hand	Condition	Cold/heat
<i>Mean noradrenaline, pg/ml (SD)</i>							
CRPS hand	56 (65)	116 (81)	35 (20)	0.75	0.99	<0.001	0.02
Contra	44 (24)	120 (66)	43 (22)				0.03
Control right	51 (17)	140 (89)	57 (14)	0.92	0.96	<0.001	0.01
Control left	57 (26)	132 (81)	55 (12)				0.02
<i>Mean skin temperature at pulps of digit two to five, °C (SD)</i>							
CRPS hand	28.2 (4.0)	25.1 (0.9)	33.1 (2.0)	0.89	0.70	<0.001	0.02
Contra	29.0 (4.0)	25.0 (1.2)	33.4 (1.9)				0.02
Control right	31.5 (2.7)	25.2 (1.6)	33.7 (1.5)	0.60	0.36	<0.001	0.008
Control left	32.5 (2.5)	25.3 (1.3)	34.5 (0.9)				0.008
<i>Mean flux at digit 1, a.u. (SD)</i>							
CRPS hand	145 (131)	36 (23)	301 (79)	0.73	0.21	<0.001	0.02
Contra	205 (132)	49 (59)	364 (119)				0.02
Control right	236 (114)	39 (33)	283 (99)	0.89	0.34	<0.001	0.01
Control left	195 (92)	24 (22)	260 (94)				0.01
<i>Mean flux at dorsum hand, a.u. (SD)</i>							
CRPS hand	29 (21)	19 (14)	96 (60)	0.84	0.67	<0.001	0.02
Contra	39 (33)	15 (5)	108 (63)				0.02
Control right	44 (34)	15 (6)	90 (50)	0.78	0.59	<0.001	0.01
Control left	32 (12)	14 (5)	58 (25)				0.01

Measurements performed during 20 min rest (baseline), during last 20 min of cooling (cold), and last 20 min of heating (heat). CRPS hand = painful hand in the patients, Contra = contralateral non-painful hand in the patients.

effect in both groups with a lower skin temperature and flux during cold compared to heat. There was no difference between the hands in the patients or the controls, and for each group, the responses in the hands were similar during baseline, cooling, and heating. Comparing patients to controls (mean skin temperature and flux of both hands), the responses were similar during baseline, cooling, and heating. For the subjective appraisals of skin temperature (Fig. 4G), there was a significant condition effect with lower experienced skin temperature during cooling compared with heating. There was no difference between the reports in patients and controls, and the responses in the groups were similar during baseline, cooling, and heating.

#### Pain ratings during thermal exposure

The mean pain rated on an NRS was 6.2 (SD 1.7) during baseline and increased to 8.5 (SD 1.8) during the last period of cooling (Fig. 4H). For all patients, the pain intensity during cooling was higher than during baseline ( $P=0.02$ ). Five of the seven patients completing the thermal exposure had a reduction in pain associated with the change from high to low sympathetic activation. The reduction to 7.2 (SD 2.5) during the last period of heating was not significant compared to that during the last period of cooling ( $P=0.24$ ). There was no correlation between changes in NE release and pain score during high versus low peripheral sympathetic activation ( $r=-0.11$ ,  $P=0.78$ ).

#### Hemodynamic changes

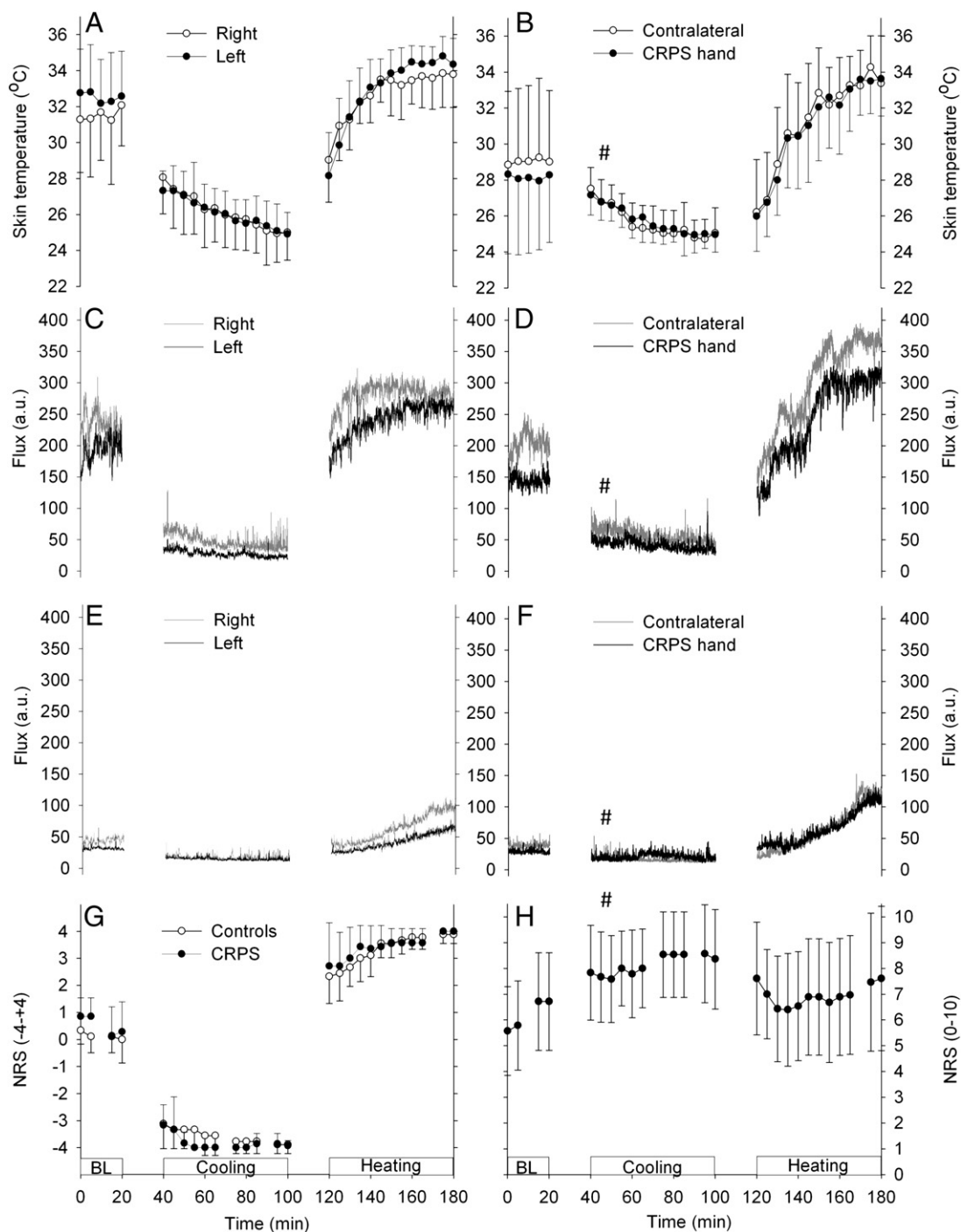
For systolic and diastolic blood pressure, there was a significant condition effect with significantly higher systolic blood pressure during cooling versus heating in patients, but not in controls (Table 2). Diastolic blood pressure was higher during cooling compared to heating in both groups. For the mean RR, there was a significant condition effect with higher mean RR values during cooling compared to heating in patients and controls.

#### Discussion

Whole-body cooling induced a significant increase in cutaneous NE release, vasoconstriction, and lowered skin temperature. The main finding in this exploratory study was that these responses were similar in CRPS patients and in healthy controls and between the affected and contralateral side in the CRPS patients. The similar magnitude of NE release in both CRPS patients and controls suggests that the evoked NE release from cutaneous sympathetic postganglionic fibres in chronic CRPS in general is preserved.

The present microdialysis study has, for the first time, measured dermal NE directly in the zone of spontaneous and evoked pain in CRPS patients. The patients and controls were exposed to cutaneous sympathetic activation (whole-body cooling) and inhibition (whole-body warming) as determined by changes in skin temperature and blood flow. The NE concentration was increased by a factor of 3 with significantly higher NE values during physiological activation of the peripheral cutaneous sympathetic fibres (cooling) compared to a low peripheral sympathetic activity during heating. The increase in NE is identical to previously reported subcutaneous NE release during cold exposure in healthy volunteers (Gronlund et al., 1994). This suggests that the dermal microdialysis with the measurement of NE is a valid method to evaluate changes in local sympathetic activity during physiological manipulations of sympathetic activity. The NE responses are dynamic, and may change with the duration of heating or cooling. In the present study, the data were plotted during the entire thermal cycle from baseline through increasing coldness and warmth (Figs. 3C and D). No differences were found. Therefore, it is unlikely that the long duration of heating and cooling could have interfered with any difference between the hands or between the patients and controls.

We used microdialysis technique to assess skin concentrations of NE, which we measured in dialysate recovered from the skin. The dialysate recovered a constant proportion of the solute in the skin known as relative recovery. It is generally accepted that relative recovery largely depends on the length and the diameter of the microdialysis membrane, the membrane material, the flow rate, the



**Fig. 4.** Skin temperature, flux, and subjectively perceived skin temperature were reduced by cooling and increased by heating. Manual skin temperature was measured at digits two to five in the controls (A) and patients (B) (mean (SD)). Mean cutaneous flux at digit one in the controls (C) and patients (D). Mean cutaneous flux near the microdialysis probe at the dorsum of the hands in controls (E) and patients (F). Subjectively perceived skin temperature (G) (mean (SD)) rated on a  $-4$  to  $+4$  numeric rating scale (NRS);  $+4$ : very hot;  $+3$ : hot;  $+2$ : warm;  $+1$ : slightly warm;  $0$ : neutral;  $-1$ : slightly cool;  $-2$ : cool;  $-3$ : cold;  $-4$ : very cold. Pain in the CRPS hand (H) rated on a NRS (0–10). Pain was not rated during the silent periods, explaining the missing NRS values after 10, 70, 90, and 170 min. # One patient was only cooled for 40 min and is not included in the time period from 40 to 60 min.

substance evaluated, and the target tissue investigated (Holmgaard et al., 2010). All but the skin composition was identical in patients and controls. The skin in the affected tissue in CRPS patients may be changed due to oedema, which in theory could influence the relative recovery. This potential issue may compromise direct comparisons of the dialysate NE concentrations during baseline in patients and controls. The effect of oedema on relative recovery in the skin has not been thoroughly investigated. Langberg et al. (1999) demonstrated

no difference in relative recovery in Achilles tendons immediately after probe insertion, after a 180 min running period and after 3 days of recovery. Also experimental studies in CRPS patients have shown that the short-lasting protein extravasation (an indicator of oedema) caused by probe implantation was identical in CRPS patients and controls in terms of peak response and elimination despite the presence of oedema in the skin of the patients but not the controls (Weber et al., 2001).



**Table 2**  
Heart rate, blood pressure, and core temperature in controls and patients exposed to cooling and heating.

	Baseline	Cold	Heat	ANOVA ( <i>P</i> value)			Wilcoxon
				Condition × group	Between-group	Condition	Cold/heat
<i>Mean RR, ms (SD)</i>							
Controls	1048 (172)	1035 (98)	850 (96)	0.93	0.35	<0.001	0.008
Patients	966 (204)	964 (239)	791 (118)				
<i>Mean systolic blood pressure, mm Hg (SD)</i>							
Controls	120 (9)	129 (10)	129 (11)	0.11	0.40	0.002	0.94
Patients	128 (17)	140 (26)	128 (13)				
<i>Mean diastolic blood pressure, mm Hg (SD)</i>							
Controls	78 (10)	88 (10)	82 (9)	0.13	0.96	0.002	0.04
Patients	83 (12)	87 (16)	79 (9)				
<i>Mean core temperature, °C (SD)</i>							
Controls	36.9 (0.4)	36.9 (0.5)	36.8 (0.5)	0.51	0.86	0.86	0.63
Patients	36.8 (0.3)	36.9 (0.4)	37.0 (0.7)				

Measurements performed during 20 min rest (baseline), during last 20 min of cooling (cold), and last 20 min of heating (heat). Mean RR interval = mean time between consecutive normal R waves in the QRS complexes.

We do realize that vascular tone may influence dialysate NE concentrations (even though relative recovery is constant). During hypoperfusion, extracellular solute concentrations may increase due to reduced washout/elimination (Boutsouki et al., 2001; Ross et al., 2006). The skin flux measured near the microdialysis probe was significantly changed during the thermal exposure. However, there was no difference in flux between the hands or between patients and controls suggesting that local flow changes were without any significance in the present comparisons. Thus, we consider it appropriate to compare baseline dialysate NE concentrations as well as temperature-dependent changes in dialysate NE concentrations among patients and controls.

We did not measure the relative recovery *in vivo*. Leis et al. (2004) performed dermal microdialysis by measuring NE at baseline and after infusion of tyramine in healthy volunteers. Their baseline NE levels of 36 pg/ml were lower than in the present study. They calculated an *in vivo* recovery of 8% using the no-net-flux method (Leis et al., 2004). We used a slower perfusion velocity (2 versus 4  $\mu$ l/min) and a longer membrane (30 versus 15 mm) with an expected higher NE recovery than 8%. A conservative approach with a recovery of 10–20% would result in baseline skin NE levels in the range 250 to 500 pg/ml. Taken together, we believe that the present method is sufficient to assess the relative changes of skin NE during thermal cooling and heating.

Overall the NE was not reduced in the present series of CRPS patients compared to controls, which is in contrast to previous findings of lower venous plasma NE in the affected CRPS limb versus the unaffected limb (Drummond et al., 1991; Harden et al., 1994; Wasner et al., 1999). However, plasma NE mainly reflects muscular sympathetic activity. Furthermore, the extraction of NE from plasma and the overflow of NE from the sympathetic nerves to the circulation (10–20%) may depend on regional changes in blood flow. Therefore, to obtain a picture of the levels of NE released in the vicinity of the dermal nociceptors in areas with evoked pain, the present intradermal microdialysis is preferable, and cutaneous and venous NE are not comparable.

It may be argued that the NE is reduced only in the acute (warm) phase of CRPS (Drummond et al., 1991). The present study included chronic CRPS patients with pain duration of more than three months. Therefore, a future identical study should compare the regulation in acute versus chronic CRPS.

We measured skin temperature and flux bilaterally in both glabrous (digits) and hairy (area of microdialysis) skin during the thermal exposure. In glabrous skin the thermoregulatory reflexes are mainly regulated by the variation in the sympathetic vasoconstrictor tone while the thermoregulatory reflexes in human hairy skin are mediated by

two branches of the sympathetic nervous system (noradrenergic vasoconstrictor nerves and cholinergic active vasodilator nerves) (Bini et al., 1980b). We have presented skin temperature data for both hairy and glabrous skin (Fig 4). The skin temperature and flux in glabrous skin reflect changes in noradrenergic sympathetic vasoconstrictor nerves and for that reason we correlated these measures to changes in NE release.

The baseline skin temperature was three degrees colder in the patients compared with the controls despite similar baseline NE levels. The correlation between baseline skin temperature and NE levels was not significant in patient or controls and the correlation coefficients were almost equal in the two groups. Thus, the NE levels were not less in the perfusate in the controls at baseline all other factors being equal. The difference in skin temperature was not statistically significant but this could be clinically meaningful. It is well known that the lowering of temperature may affect nerve function, e.g. conduction velocity (Buchthal, 1991).

The pain reduction was not correlated with a reduction in NE release. However, for all patients, the pain intensity during cooling was higher than during baseline, and five of the seven patients who completed the thermal exposure had reduced pain scores when moving from high to low peripheral sympathetic activation. This is a secondary outcome but is compatible with the notion that NE released from sympathetic fibres may cause pain as observed in conditions termed sympathetic maintained pain (Price et al., 1998; vanEijs et al., 2012). The present study did not include a control group with pain other than CRPS and cannot rule out whether the thermal induced changes in pain are unique to CRPS or do occur in other pain patients.

The systolic blood pressure in the patients and the diastolic blood pressure in both groups were higher and the heart rate was lower in both groups during whole-body cooling than during whole-body heating. Therefore, cooling and heating not only modulated the cutaneous sympathetic fibres, but it most likely also modulated sympathetic fibres innervating vessels in the skeletal musculature as well as efferent cardiac sympathetic and parasympathetic fibres. Therefore, the higher pain in the patients during cooling may also be related to sympathetic activation of deeper somatic tissues. There seem to be further mechanisms apart from NE in which sympathetic neurons are involved in nociception (Green et al., 1997). We did not test for other possible explanations for sympathetic mediated pain in CRPS, such as changes in the co-release of other substances together with NE, reduced degradations of transmitters in the milieu of the sensory nerves due to reduced regional cutaneous flow, or sensitisation of

cutaneous nociceptive C fibres to adrenergic excitation. In our cohort, the evoked NE release from cutaneous sympathetic postganglionic fibres was in general preserved suggesting that the NE levels are not the culprit in the sympathetic mediated pain. In line with this recent evidence rather points to a sensitization of adrenergic receptors than to an increased efferent sympathetic activity. Particularly the sensitization of alpha 1-adrenoceptors on cutaneous nociceptive C-fibres may play a major role independent of the NE levels (Campbell et al., 1992; Dawson et al., 2011). It is possible that additional information would have been obtained if skin punch biopsies had been carried out to study a change in alpha1-adrenoceptor density in affected skin versus control skin (Drummond et al., 1991) and quantification of sudomotor and sensory nerves would have provided additional evidence.

The core temperature was not changed in the two groups during cooling and heating as previously described (Baron et al., 2002). This suggests that the participants had effective mechanisms to regulate heat loss and heat production and to maintain body temperature within a limited range. An increased core temperature is known to induce significant increases in plasma NE (Rowell, 1990; Wright et al., 2010) underlining the importance of an unchanged core temperature in the present set-up modulating cutaneous sympathetic activity.

## Conclusion

This study is the first to measure cutaneous NE directly in the zone of evoked pain during physiological modulation of the peripheral sympathetic nervous system with whole-body cooling and heating in CRPS patients. No differences in cutaneous NE were found in patients and controls or between the affected and unaffected CRPS hand, suggesting that the evoked NE release from cutaneous sympathetic postganglionic fibres in CRPS is preserved in chronic CRPS. The study calls for additional studies in which acute and chronic CRPS is taken into consideration.

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