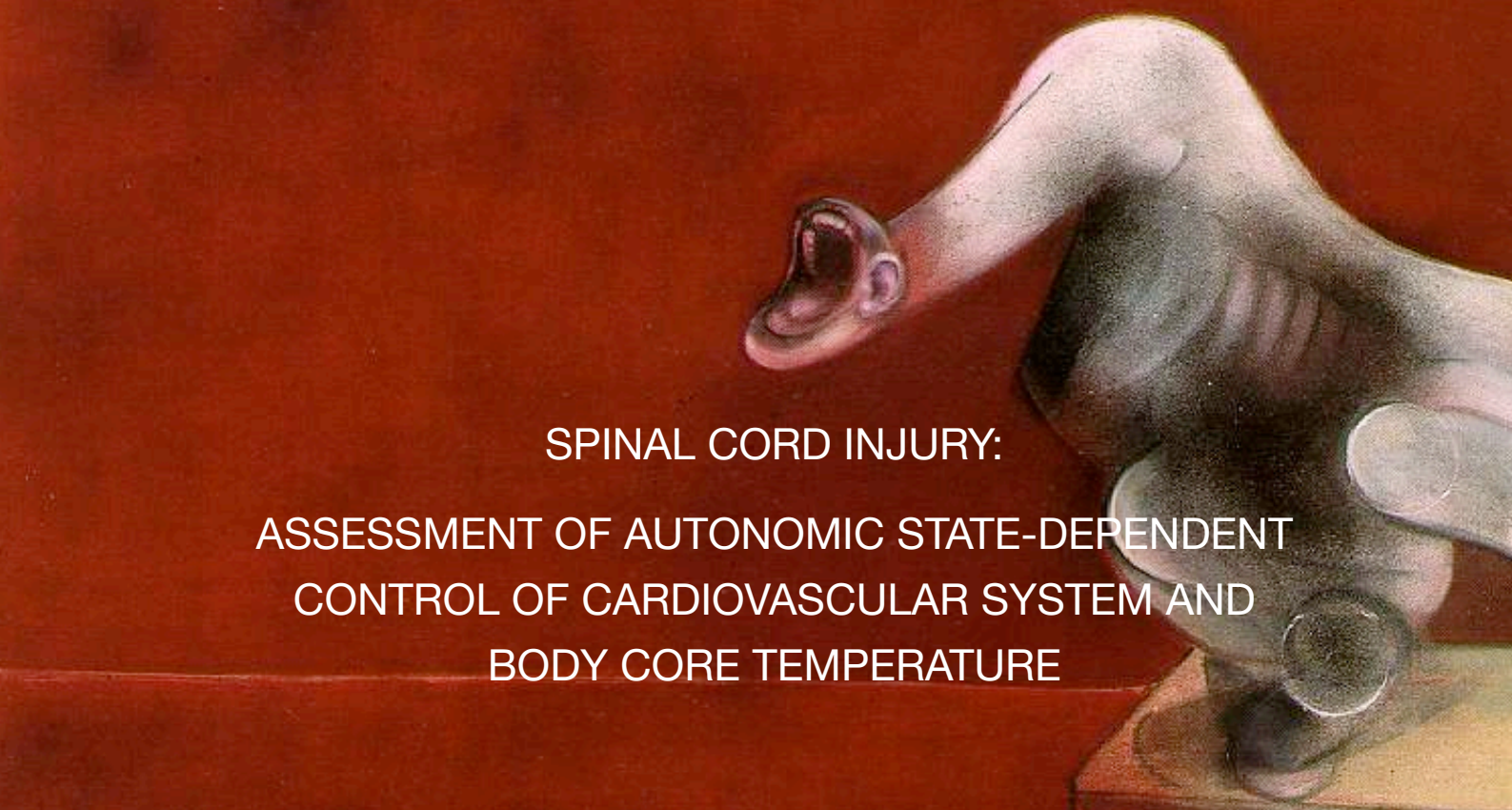
Alma Mater Studiorum – Università di Bologna
Dottorato in Scienze Mediche Specialistiche

DOTTORATO DI RICERCA IN MEDICINA DEL SONNO

XXIV Ciclo



SPINAL CORD INJURY:

ASSESSMENT OF AUTONOMIC STATE-DEPENDENT CONTROL OF CARDIOVASCULAR SYSTEM AND BODY CORE TEMPERATURE

Presentata da: Dr. Pietro Guaraldi

Coordinatore Dottorato:

Prof. Pietro Cortelli

Relatori:

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Dr.ssa Federica Provini

Anno 2012

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DOTTORATO DI RICERCA IN SCIENZE MEDICHE SPECIALISTICHE

Progetto n°2 “MEDICINA DEL SONNO”

XXIV Ciclo

Settore Concorsuale: 06/D6 - NEUROLOGIA

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(n° matr. 376057)

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Esame finale anno 2012

ad Achira (Chiara)

"To whom I owe the leaping delight

That quickens my senses in our wakingtime

And the rhythm that governs the repose of our sleepingtime,

the breathing in unison"

T. S. Eliot

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LIST OF ABBREVIATIONS

Δ (delta): Change of any variable quantity

ΔBcT: changes in BcT

ΔDBP: changes in DBP

ΔHR: changes in HR

ΔSBP: changes in SBP

ACR: acrophase

AD: autonomic dysreflexia

AHI: apnea hypopnea index

AMP: amplitude

ANOVA: analysis of variance

ANS: autonomic nervous system

ASIA: American Spinal Injury Association

BcT: body core temperature

BMI: body mass index

BP: blood pressure

C4-7: cervical SCI

CF: cold face

CHD: coronary heart disease

CNT: control subjects

CPG: central pattern generators

CVD: cardiovascular disease

D2-12: thoracic SCI

DB: deep breathing

DBP: diastolic blood pressure

EEG: electroencephalogram

HF: high frequency

HR: heart rate

HRV: heart rate variability

HUTT: head-up tilt test

IE: isometric exercise

LF: low frequency

MET: metabolic equivalent

N1: stage 1 NREM sleep

N2: stage 2 NREM sleep

N3: stage 3 NREM sleep

NREM: non REM

NREM: non-rapid eye-movement sleep

OSA: obstructive sleep apnea

OV: overshoot

PLMS: periodic limb movement during sleep

PS: parasympathetic

PSA: power spectral analysis

PSG: polysomnographic

REM: rapid eye-movement sleep

SBP: systolic blood pressure

SCI: spinal cord injury

SE: sleep efficiency

SNA: sympathetic nerve activity

SRBD: sleep related breathing disorders

SSR: sympathetic skin response

SWS: slow wave sleep

TP: Total Power

TPR: total peripheral resistance

TST: total sleep time

V-PSG: Video-Polysomnography

VLF: very low frequency

VM: Valsalva manoeuvre

VR: Valsalva ratio

W: wake

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INTRODUCTION

"The frog instantly dies when the spinal cord is pierced, and previous to this it lived without head, heart or any bowels or skin, and here, therefore, it would seem lies the foundation of movement and life."

Leonardo da Vinci
(Quaderni d'Anatomica, Vol. V)

INTRODUCTION

Scientific Background and Object of the Study:

Few survivable injuries have as much impact on a patient's life as acute spinal cord trauma and its associated human and social cost. Spinal cord injury (SCI) results not only in paralysis; but it is also associated with a range of autonomic dysregulation that can interfere with cardiovascular, bladder, bowel, temperature, and sexual function. The entity of the autonomic dysfunction is related to the level and severity of injury to descending autonomic (sympathetic) pathways [1-3].

For many years there was limited awareness of these issues and the attention given to them by the scientific and medical community was scarce. Yet, even if a new system to document the impact of SCI on autonomic function has recently been proposed [4], the current standard of assessment of SCI (American Spinal Injury Association (ASIA) examination) evaluates motor and sensory pathways, but not severity of injury to autonomic pathways [5].

Beside the severe impact on quality of life, autonomic dysfunction in persons with SCI is associated with increased risk of cardiovascular disease and mortality [3,6,7]. Therefore, obtaining information regarding autonomic function in persons with SCI is pivotal and clinical examinations and laboratory evaluations to detect the presence of autonomic dysfunction and quantitate its severity are mandatory.

Furthermore, previous studies demonstrated that there is an intimate relationship between the autonomic nervous system and sleep from anatomical, physiological, and neurochemical points of view [8]. Although, even if previous epidemiological studies demonstrated that sleep problems are common in SCI [9-12], so far only limited polysomnographic (PSG) data are available [13,14]. Finally, until now, circadian and state dependent autonomic regulation of blood pressure (BP), heart rate (HR) and body core temperature (BcT) were never assessed in SCI patients.

Aim of the current study was to establish the association between the autonomic control of the cardiovascular function, thermoregulation, sleep parameters and increased cardiovascular risk in SCI patients.

Study Population and Main Parameters:

Thanks to the collaboration with the Spinal Unit of Azienda Ospedaliera Carreggi, Florence, ITALY, we assessed 5 cervical SCI with tetraplegia (C4-C7 lesions), 7 thoracic SCI with paraplegia (D2-D12 lesions). All patients underwent the following studies:

I. Nocturnal cardio-respiratory monitoring for detection of sleep related breathing disorders;

II. Assessment of daytime autonomic control of the cardiovascular system through cardiovascular reflexes;

III. Assessment of heart rate variability (HRV) during supine rest and head-up tilt;

IV. Evaluation of Sleep structure and efficiency by means of video-PSG;

V. Assessment of circadian rhythm and state and time dependent regulation of BP and HR;

VI. Assessment of circadian rhythm and state and time dependent regulation of BcT.

Patients results were compared to controls, whose number varied according to the different assessments.

Inclusion Criteria:

- Male sex;
- Complete spinal cord lesion (Frankel A);
- Clinically stable (at least 6 months after the spinal cord lesion);
- Absence of symptoms or signs of diabetes, cardiorespiratory disease or of other pathological conditions that might affect autonomic cardiovascular control;
- Patient provided informed consent to participate to the study.

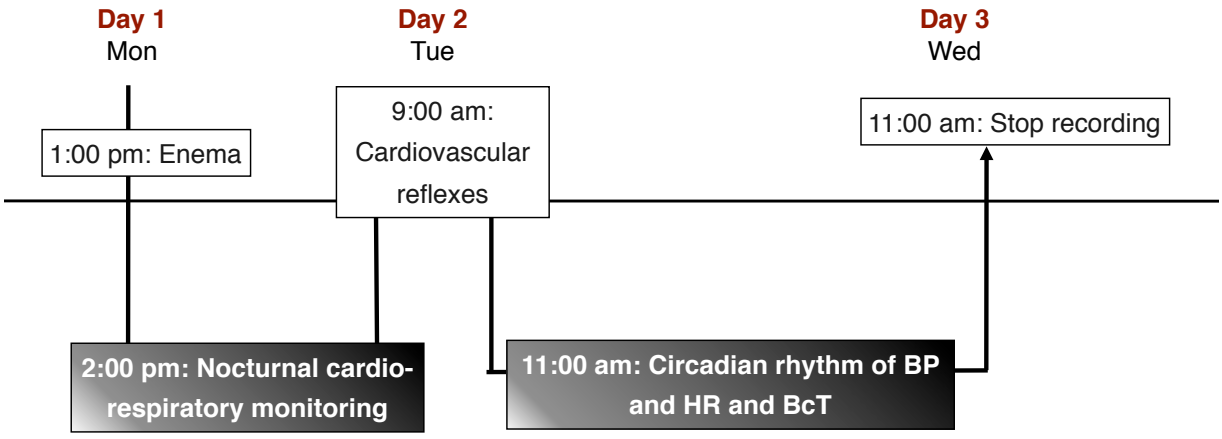
Study Protocol

Patients and controls were admitted to hospital and studied following a 3-days protocol (Fig 0.1). Subjects were admitted on Monday morning; in the afternoon they received an enema to prepare for rectal temperature monitoring and at night they underwent nocturnal cardio-respiratory monitoring for detection of sleep related breathing disorders;.

On the following morning, between 8:00 am and 11:00 am, subjects underwent cardiovascular reflexes tests in a temperature controlled (23±1°C) clinical investigation room according to standard procedures [15].

After the assessment of cardiovascular reflexes (between 11:00 am and 12:00 am), arterial BP, HR, BcT and sleep-wake cycle were continuously monitored for 24 hours under controlled conditions (recording ending at 11:00 am of the following day).

Fig 0.1: study protocol



Thesis Structure

The thesis is organized in Sections originating from the original study protocol.

In each module it is specified the included population of patients and controls, the methods of data collection and analysis, results, discussion and conclusions.

The sections are structured as follow:

I. Sleep structure in spinal cord injury patients

II. Analysis of cardiovascular parameters

1. Autonomic control of the cardiovascular system during wakefulness
2. The circadian rhythm of blood pressure and heart rate in spinal cord injury patients

III. Body core temperature regulation in spinal cord injury patients

In the Appendix figures, which were reduced in size to facilitate reading in each section, are reported in higher magnification, together with additional supplementary material.

The thesis is written in English to allow international diffusion and to favour its publication on peer reviewed medical journal.

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Section I

SLEEP STRUCTURE IN SPINAL CORD INJURY PATIENTS

“Something for the rag and bone man
Over my dead body
Something big is gonna happen
Over my dead body
Someone saw someone's daughter
Over my dead body
This is how I ended up sucked in
Over my dead body
I'm gonna go to sleep
And let this wash all over me
We don't really want a monster taking over
Tip toeing, tying down
We don't want the loonies takin' over
Tip toeing, tying down our arms
May pretty horses
Come to you as you sleep
I'm gonna go to sleep
And let this wash over me”

Radiohead
(Go to Sleep, Hail to the Thief, 2003)

Section I: SLEEP STRUCTURE IN SPINAL CORD INJURY PATIENTS:

A 24-h study under bed rest controlled conditions

Background:

Previous cross-sectional descriptive studies and epidemiological reviews [1-4] demonstrated that sleep problems are common in spinal cord injury (SCI). These studies, which are based on sleep scales and self-administered questionnaire, demonstrated that patients with SCI, in comparison to the normal population, have greater difficulty falling asleep, have more frequent awakenings, sleep subjectively less well, sleep more hours, take more and longer naps, and snore more [4]. The elevated 15–40% prevalence of sleep disorders in SCI individuals [1,3,5,6] is higher than the ~4% prevalence typically reported in middle-aged men [7] and were related to: a) direct consequences of the injury, like pain and muscle spasm; b) concomitant sleep disorders, such as sleep related breathing disorders (SRBD) or periodic limb movement during sleep (PLMS); or c) to less specific associated condition as anxiety and depression.

Most of the study on sleep in SCI patients focused on SRBD demonstrating an increased incidence of sleep apnea [5,8-13]. Back in 1995, McEvoy et al. demonstrated, by means of a overnight cardio-respiratory monitoring on 40 tetraplegic patients, that the prevalence of SRBD is more than twice that observed in normal population [8]. Eleven out of 40 patients had an apnea hypopnea index (AHI) >15 per hour. More than 80% of apneas were obstructive or mixed, with no sustained hypoventilation. The AHI was found to correlate with neck circumference and sleeping position, but not with respiratory function, level of lesion or dose of antispastic medications. Further studies confirmed that SCI and tetraplegics in particular, have a higher incidence of SRBD [4,14,15], but provided conflicting results on the mechanisms contributing to sleep apnea [13]. The pathophysiology of SRBD in SCI is yet not fully understood. Several different predisposing factors may be involved: circumstances related to the injury, like paralyzed intercostal and abdominal muscles, impaired activation of diaphragm (in lesions above C5), or generic risk factors for SRBD, such as obesity, neck circumference, increased upper airways resistance and supine sleep posture. Furthermore, medications, such as benzodiazepines and baclofen, having relaxant effect on breathing muscle and upper airways together with a depressant effect on the central nervous system, may be involved [8-12,16-18].

Despite the previously mentioned epidemiological studies and the plethora of studies on SRBD, so far only limited data are available on sleep architecture in SCI. In 1968 Adey et al. [19] assessed by means of polysomnography (PSG) 8 upper cervical and 2 upper thoracic SCI patients in the early post-lesion period and 6 upper cervical and 1 lower thoracic SCI patients in a late post-lesion period. SCI patients in early post lesion period had a reduced total sleep time (TST) and an abnormal representation of sleep phases (a reduction in slow wave sleep (SWS) & REM sleep). Instead, SCI patients in the late post lesion period, demonstrated only a high prevalence of light sleep. No relation between age at onset and sleep distortion was found, but the patient with thoracic SCI, had a proportion of non REM (NREM) sleep and REM sleep similar to controls. Similarly, in a more recent polysomnographic case-controlled preliminary study by Scheer et al. [20] on a small sample of patients (3 cervical and 2 thoracic SCI), tetraplegics had a significantly lower (83%) sleep efficiency (SE) compared to thoracic SCI (93%). The SE of thoracic SCI was not different from healthy control subjects (94%). Moreover, there was no difference in the proportion of the different sleep stages, but cervical SCI had a significantly increased REM-onset latency compared to subjects with thoracic SCI.

Objective:

Aim of the study was to assess with a 24-h Video-Polysomnography (V-PSG) the sleep structure in a population of 5 cervical SCI with tetraplegia, 7 thoracic SCI with paraplegia and 7 age and sex matched control subjects.

Materials and Methods:

Subjects

Five cervical SCI with tetraplegia (C4-7) and 7 thoracic SCI with paraplegia (D2-12) and 7 control subjects (CNT) underwent a 24-h V-PSG under bed-rest controlled conditions (Table I.1). One thoracic SCI (Pt 12) and all controls prolonged the V-PSG recording for further 24-h. Prior to data collection, all subjects were questioned regarding SRBD symptoms, and underwent a clinical examination to exclude pathological conditions that might affect sleep-wake cycle. All patients (excluding patient 1 and 4) and all controls were assessed for sleep related breathing disorders by means of a previous nocturnal cardio-respiratory monitoring.

The investigation conformed with the principles outlined in the Declaration of Helsinki [21]. The protocol was approved by the Institutional Review Board of the University of Bologna and all participants provided informed consent.

Study protocol

Wake-sleep cycle were continuously monitored for 24-h by an ambulatory polygraphic recorder (Albert Grass Heritage®, Colleague TM PSG Model PSG16P-1, Astro-Med, Inc, West Warwick, RI, USA or Neurofax Electroencephalograph EEG-1200, Nihon-Kohden Corp., Tokyo, Japan) recording electroencephalogram (EEG: C3-A2, C4-A1, O2-A1), right and left electrooculogram, electrocardiogram, and electromyogram of the mylohyoideus, left and right anterior tibialis muscles and thoraco-abdominal breathing. Simultaneous video monitoring was performed throughout the recording to assist in off-line data interpretation. During the study subjects were allowed to sleep ad libitum, living in a temperature (24±1°C) and humidity (40-50%) controlled room, lying in bed except when eating, in a light-dark schedule (light-off period: 11:00 pm-7:00 am). The subjects were placed on a 1.800 kcal/day diet divided into three meals (8:00 am, 12:00 am, 6:00 pm) and three snacks (10:00 am, 4:00 pm, 11:00 pm). From midnight preceding the monitoring, subjects were instructed to avoid alcohol and caffeinated beverages and to abstain from smoking.

Data analyses

Patients were considered “underweight” if BMI was below 18,5; “normoweight” if BMI was between 18,5 and 24,9; “overweight” if BMI was between 25 and 29,9; “obese” if BMI was 30 or greater.

Sleep-related breathing disorders were diagnosed according to the criteria of the American Academy of Sleep Medicine Task Force [22] and rated as “mild” if the AHI was between 5 and 15; “moderate”, if AHI was between15 and 30 or “severe” if the AHI was greater than 30.

The 24-h sleep-wake cycle starting from 11:00 am on the first day of PSG recording was visually scored in 30 s epochs according to the standardized criteria of Rechtschaffen and Kales [23] as light (stages N1 and N2) NREM sleep, deep (stage N3) NREM sleep, and REM sleep. The total sleep time (TST), the sleep efficiency (SE: time spent asleep/time in bed x 100), the duration (min) and the percentage (%) of TST of each NREM sleep stage (N1, N2, N3) and of REM sleep, were calculated for every subject over the light-off period (from 11:00 pm to 7:00 am).

The arousal index (AI: number of arousals/hour of sleep), the periodic limb movements in sleep index (PLMS-I: number of PLMS/hour of sleep) and the PLMS-arousal index (PLMS-AI: number of PLM associated with arousal/hour of sleep) were computed over the light-off period according to the scoring rules of the Sleep Disorders Atlas Task Force of the American Sleep Disorders Association [24] and the American Academy of Sleep Medicine Manual for the scoring of Sleep [25] respectively. An AI>15 and a PLMS-I>5 were considered as abnormal.

Wake-sleep fragmentation was determined by calculating the frame shifts index indicating the number of 30 s sleep stage shifts occurring every 15 minutes throughout the light-off period (from 11:00 pm to 7:00 am).

Statistical analysis

Data from sleep variables not normally distributed, were compared between groups using the Kruskal–Wallis test. All statistical analyses were performed with IBM SPSS Statistics version 20.0 and a $p<0,05$ was considered significant.

Table I.1 Patients clinical characteristics and medical therapy

Pt n°	Group	Lesion Site	Age (yrs)	Dis Hx (yrs)	BMI	SRB symptoms	Therapy / day
1	C4-7	C4	32	15	24	habitual snoring, apneas, daytime sleepiness	- baclofen 100 mg - tamsulosin hydrochl 0,4 mg
2	C4-7	C4-C5	44	11	32	nil	- risperidone 2mg - bisacodyl 10 mg - triazolam 125 mcg - lactulose
3	C4-7	C5-C6	60	25	23	habitual snoring, apneas	- dantrolene sodium 75 mg - valproic acid 500 mg - lorazepam 2,5 mg - baclofen 75 mg - terazosin 5 mg
4	C4-7	C5-C6	16	2	23	nil	- sertraline hydrochl 50 mg
5	C4-7	C6-C7	54	1	22	habitual snoring, apneas, daytime sleepiness	- dihydroergotamine 2mg/ml 15 - tamsulosin hydrochl 0,4 mg - desmopressin 60 mcg - dalteparin sodium 5000 U - gabapentin 600 mg - baclofen 50 mg - oxybutynin 10 mg - citalopram 20 mg - lansoprazole 15 mg
6	D2-12	D2-D3	24	4	18	nil	- baclofen 75 mg - pregabalin 300 mg - trospium chloride 40 mg - lansoprazole 30 mg - clomipramine 25 mg
7	D2-12	D4	59	1	29	habitual snoring in supine position	- hydroclorotiazide 25 mg - carvedilol 12,5 mg - amlodipine 5 mg - ramipril 5 mg - amiloride 2,85 mg - citalopram 10 mg
8	D2-12	D4-D5	34	1	20	nil	- baclofen 75 mg - oxybutynin 15 mg - pregabalin 150 mg - tizanidine 3 mg
9	D2-12	D7-D8	26	0,5	23	nil	- oxybutynin 10 mg - etidronic acid 1200 mg
11	D2-12	D9	61	1	23	habitual snoring, apneas	- triazolam 125 mcg - paroxetine 20 mg - enoxaparin sodium 6000 U - oxybutynin 15 mg - diltiazem 30 mg - lansoprazole 30 mg - tizanidine 18 mg
12	D2-12	D10	31	3	31	nil	- intrathecal baclofen 0,5 mg - intrathecal morphine 1,5 mg
13	D2-12	D12	44	1,5	30	habitual snoring in supine position	– nil

BMI=body mass index; Dis Hx=disease history; Pt n°=patient number; SRB symptoms=symptoms of sleep related breathing disorders; yrs=years.

Results:

Subjects characteristics

Patients clinical characteristics and medical therapy are listed in Table I.1. All subjects were males. Cervical SCI ranged from C4 to C7. Thoracic SCI ranged from T2 to T12. Mean age±SD was 41±18 years for tetraplegics, 41±15 years for paraplegics and 46±11 years for controls (Table I.2). All patients had a complete Frankel A spinal cord lesion and they were clinically stable (the traumatic event occurred more than 6 months before the

time of the study). All SCI patients had a traumatic lesion, with the exception of patient 7 who suffered a spinal cord ischemic lesion. Cervical SCI had a disease history of 11±10 years (min-max=1-25), while in thoracic SCI ranged from 0,5 to 5 years with a mean of 2±1 years.

There were no age statistically significant differences between groups (Table I.2).

Among the 5 cervical SCI patients 4 were normoweight and 1 patient (Pt 2) was obese. In the thoracic SCI, 1 patient was underweight, 3 normoweight, 2 overweight and 1 obese. In the controls 1 patient was normoweight, 2 were overweight and 4 obese. Even if controls compared to SCI patient had a higher BMI, there were no statistically significant differences in BMI between groups (Table I.2).

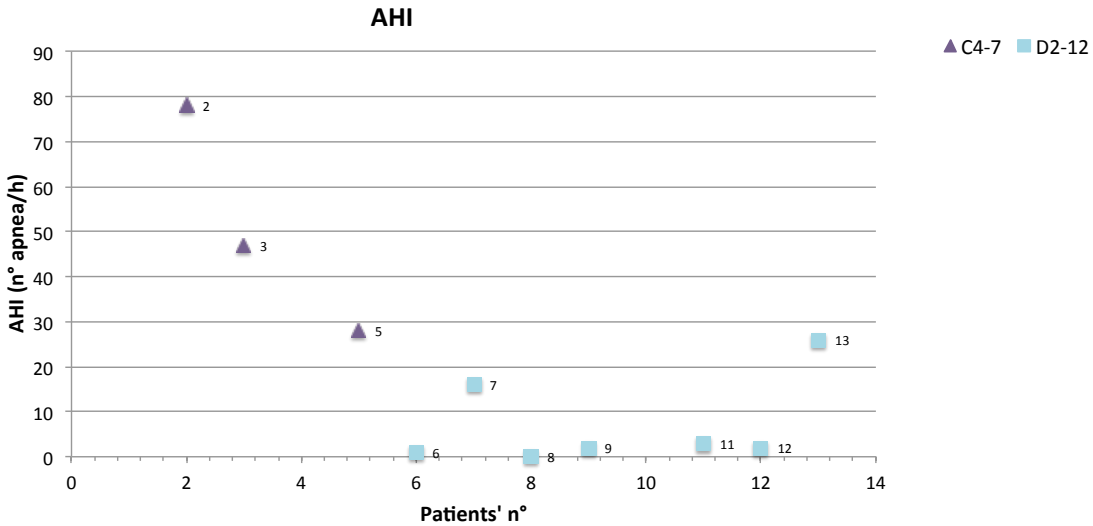
Three (Pt 1, 3 and 5) out of 5 cervical SCI patients reported apneas during sleep, 2 thoracic patients reported habitual snoring in supine position (Pt 7 and 13) and 1 thoracic patient (Pt 11) reported sleep apneas with no daytime sleepiness (Table I.1). The nocturnal cardio-respiratory monitoring conformed that 2 (Pt 2 and 3) out of the 3 cervical SCI patients had a severe obstructive sleep apnea (OSA), while 1 (Pt 5) had a moderate OSA (Fig I.1). Due to the limitations deriving from the many complex issues related to SCI patient handling and time constraint, patient 1, who was symptomatic for SRBD, and patient 4 did not perform the nocturnal cardio-respiratory monitoring. Among the thoracic SCI group the nocturnal cardio-respiratory monitoring revealed that 2 patients (Pt 7 and 13) had a moderate OSA (Fig I.1). The remaining paraplegic patients had a AHI<5. All controls, despite their higher BMI, had a AHI<10 and reported no SRBD disturbances (with the exception of positional snoring). Overall, cervical SCI had a significantly higher AHI and a higher prevalence of obstructive sleep apnea compared to thoracic SCI and controls.

Table I.2: Age and body mass index (BMI) in SCI patients and controls

	CNT (n=7)	C4-7 (n=5)	D2-12 (n=7)
Age (years)	46±11	41±18	40±15
BMI	28,97±4,04	23,77±2,51	24,84±4,97

Values are means ± SD.

Fig I.1 Apnea hypopnea index (AHI) in SCI patients



C4-C7: cervical SCI; D2-D12: thoracic SCI; SCI: spinal cord injury. Numbers on axis of abscissas correspond to patients' numbers.

Sleep parameters

Sleep variables over the night-time (from 11:00 pm to 7:00 am) are shown in Table I.3, Fig I.2, Fig I.3 and Fig I.4. There were no significant differences in TST and SE between the three groups (Table I.3).

Cervical SCI compared to controls had a significantly lower amount of N1 NREM sleep (p=0,01), while no differences were observed between thoracic SCI and CNT (Table I.3). Cervical and thoracic SCI had a significantly higher amount of N2 NREM sleep compared to controls (p=0,008 and p=0,000, respectively) while the amount of stage 3 NREM seep was significantly reduced in tetraplegics and paraplegics compared to controls (p=0,039 and p=0,002, respectively) (Table I.3). Noteworthy, 1 cervical SCI patient (Pt 2) and two thoracic SCI (Pt 11 and 12) did not reach stage 3 NREM sleep during the night-time. REM sleep was equally present in all the 3 groups, but one cervical SCI (Pt 2) and one thoracic SCI (Pt 8) presented no REM sleep stages during the light-off period (Fig I.3). The analysis of the percentages of sleep phases out of TST (Fig I.2) confirmed a higher prevalence of N2 NREM sleep and a reduced percentage of SWS in cervical and thoracic SCI compared to controls.

Table I.3: sleep parameters over the night-time in SCI patients and controls

	CNT (n=14)	C4-7 (n=5)	D2-12 (n=8)
AHI	<10*	51±25	7±10*
TST (min)	345±84	388±97	386±92
Sleep Efficiency (%)	72±17	74±17	71±16
N1 (min)	22±20*	5±5	9±5
N2 (min)	157±48*,§	275±56	283±62
N3 (min)	89±30*,§	49±28	34±42
REM (min)	80±26	59±39	59±34
N° of Arousals	56±34*	177±95	115±117
Arousal index	10±5	32±24	18±18
N° PLMS	29±48	176±393	210±312
PLMS index	5±7	25±56	38±60
N° of arousals related to leg movement	9±13	22±43	34±61
Arousal related to leg movement index	2±2	3±6	6±10
N° of phase shift	99±24	135±102	152±58
Phase shift index	18±6	25±23	25±14

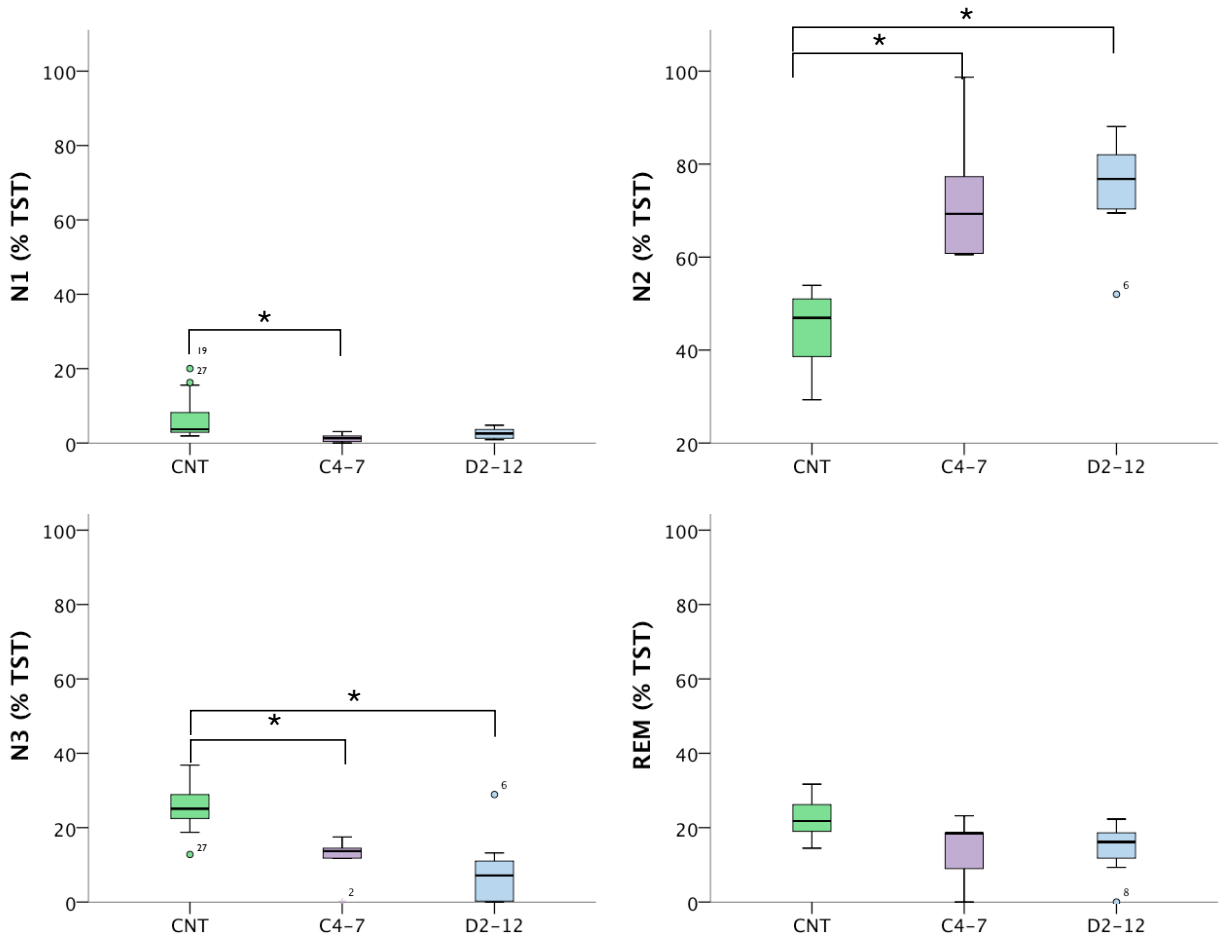
Values are means ± SD. =p<0,05 vs. cervical SCI. §=p<0,05 vs. thoracic SCI.

The total number of arousal was significantly higher in cervical SCI compared to controls (p=0,022*)* but no statistically significant differences were observed between thoracic SCI and controls, nor in the arousal index between the 3 groups (Table I.3, Fig I.4).

The analysis wake-sleep fragmentation (based on the number of phase shift and phase shift index) over the night-time-period, due marked intra-subjects variability, disclosed no statistically significant differences between the 3 groups (Table I.3; Fig I.4).

Only 1 cervical SCI (Pt 3), 2 thoracic SCI (Pt 11 and 12) and 2 controls (subjects 17 and 19) had a PLMS Index > 5 (Fig I.5). The PLMS index was markedly elevated in Pt 3, 11 and 12 (PLMS-I=124; 66; 80, respectively) while it was 13 and 9,5 in subjects 17 and 19 (Fig I.5). Due to this intra-subjects variability, despite the mean value of PLMS and PLMS-I appeared higher in SCI subjects, no statistically significant differences were observed between the 3 groups (Table I.3; Fig I.4).

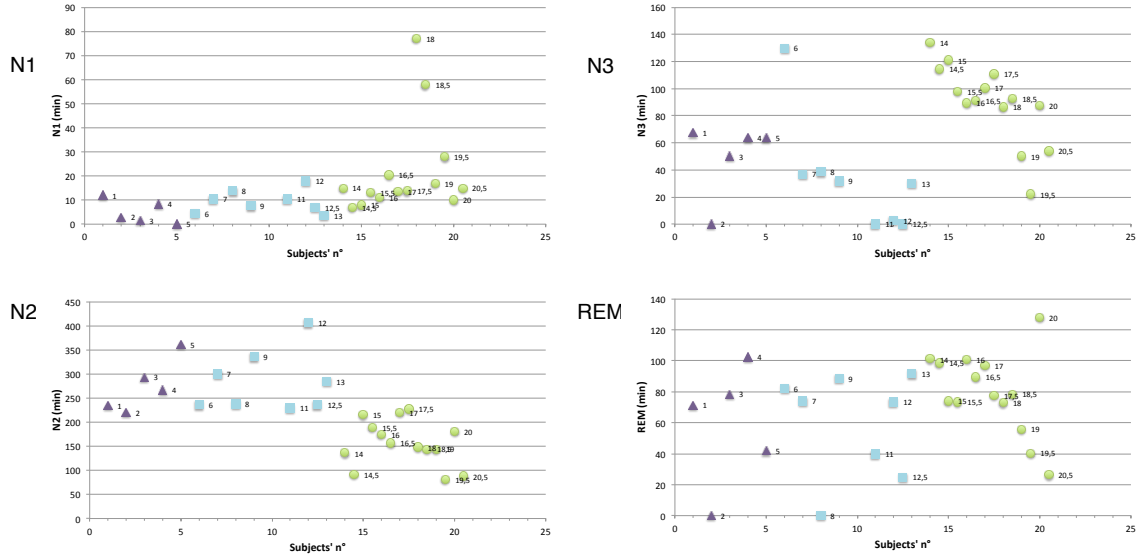
Fig I.2: Percentage of TST occupied by NREM and REM sleep phases in SCI patients and controls



*=p<0,05

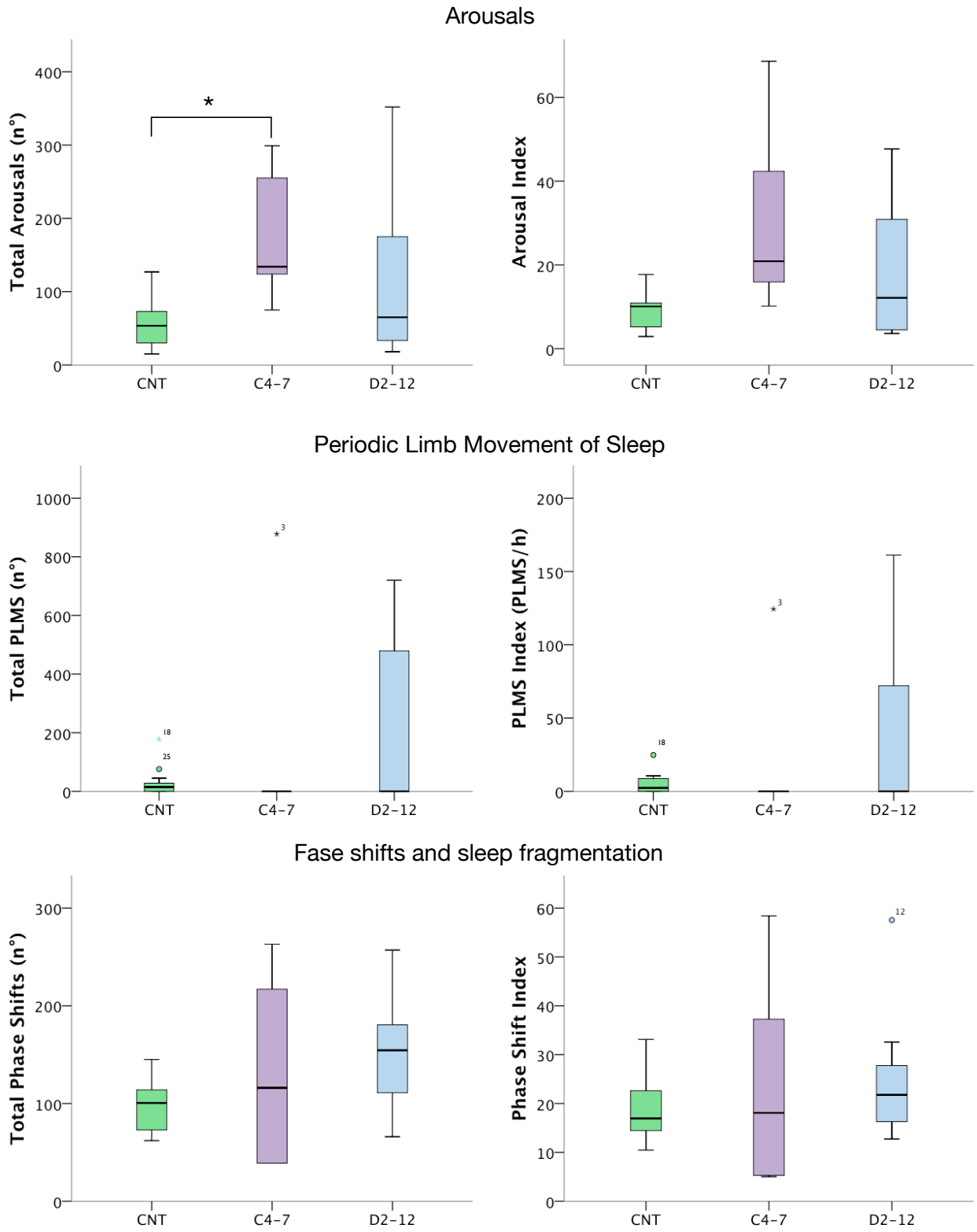
C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury, N1: stage 1 NREM sleep; N2: stage 2 NREM sleep; N3: stage 3 NREM sleep; REM: REM sleep; SCI: spinal cord injury;TST: total sleep time.

Fig I.3: minutes of NREM and REM sleep phases in SCI patients and controls



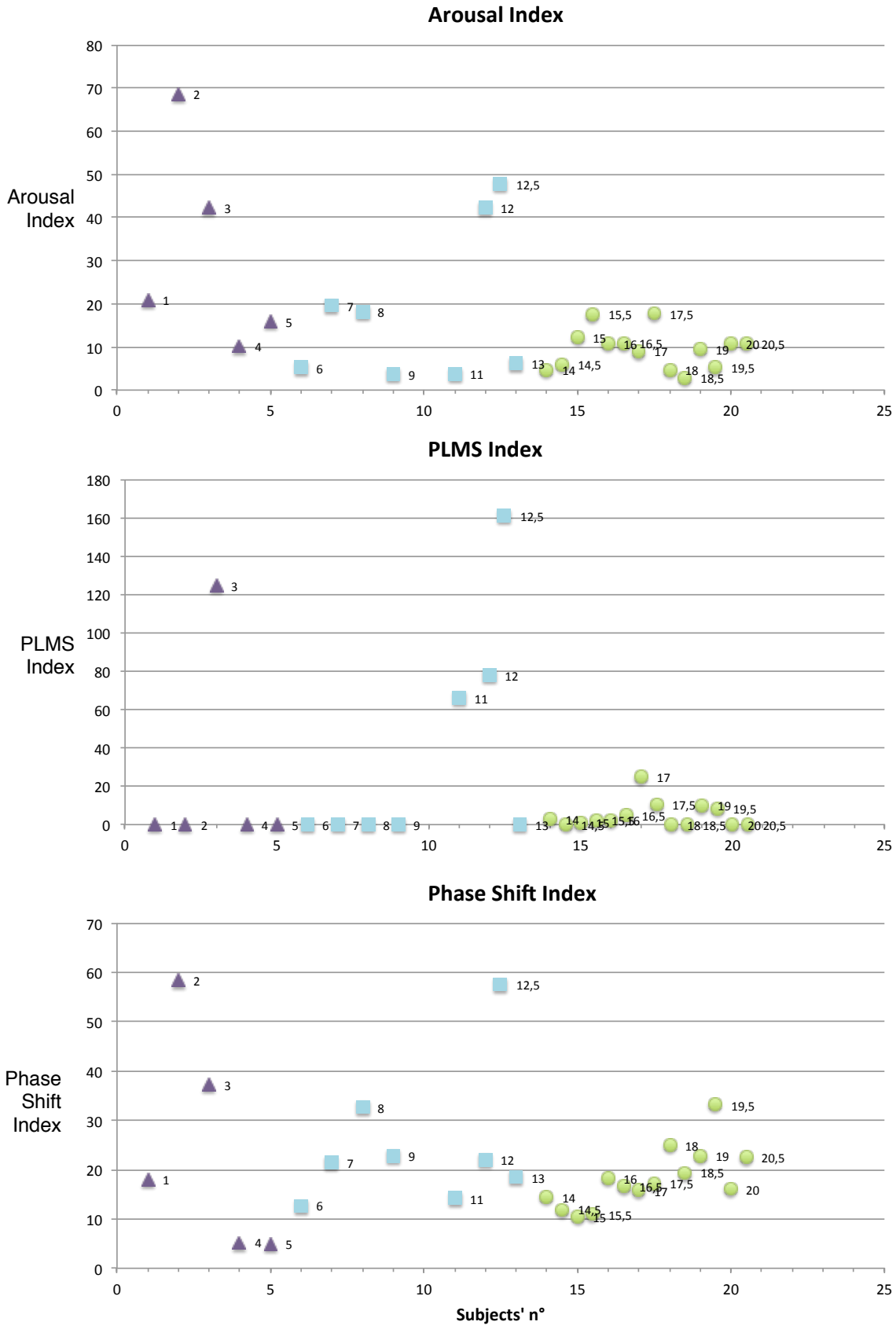
▲ C4-7 ■ D2-12 ● CNT Numbers on axis of abscissas correspond to subjects' numbers. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury..

Fig I.4: arousals, periodic limb movement and phase shifts in SCI patients and controls



∗=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury, PLMS: periodic limb movement during sleep.

Fig I.5: arousals, periodic limb movement and phase shifts in SCI patients and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury, PLMS: periodic limb movement during sleep. Numbers on axis of abscissas correspond to subjects' numbers.

Discussion:

This study investigated the sleep structure and the phase shifts in 5 cervical SCI, 7 thoracic SCI and 7 healthy control subjects following a 24-h schedule of bed-rest under controlled condition, at least 6 month after the SCI. So far, this study represent the largest V-PSG study in chronic SCI patients ever published.

The results showed that there were no significative differences in TST and SE among the 3 groups. Although, cervical and thoracic SCI patients presented a significantly higher amount of N2 NREM sleep and a significant reduction in SWS.

Our results are in line with previous findings form Adey et al. [19] who found that in the late post-lesion period 6 upper cervical patients demonstrated a high prevalence of light sleep (78±9,6% of TST), but no other abnormalities. In this previous study only 1 patients with chronic lower thoracic SCI was assessed, and presented a proportion of NREM sleep and REM sleep similar to heathy subjects [19]. Instead, the 7 thoracic SCI we assessed had an increase in light seep and a reduction in SWS similar to tetraplegics. Compared to the preliminary results by Scheer et al. [20] on 3 cervical and 2 thoracic SCI, they did not find any differences in the proportion of the different sleep stages in tetraplegics, paraplegics and controls as we did. Furthermore, they observed a significantly lower sleep efficiency in cervical SCI compared to thoracic SCI and controls. However, the number of patients they assessed is too exiguous to draw any conclusion, and the small sample size could explain the discrepancies between our results and theirs.

No differences were found in the amount of REM sleep between the three groups. In humans, high cervical lesions interrupting continuity between the medulla and spinal cord have been shown to disrupt spinal innervated REM atonia, but to preserve other characteristics of REM sleep, localizing the neural structures generating REM sleep rostral to the spinal cord, but caudal to the midbrain [26]. Even though, all our SCI patients (including high cervical SCI patients, i.e. Pt 1 who had a C4 lesion) who reached REM sleep, preserved the standard atonia.

Noteworthy, 1 cervical (Pt 2) and 2 thoracic SCI patients (Pt 11 and 12) did not reach stage 3 NREM sleep. Patient 2 and 12 were obese (BMI=32 and 31 respectively). Pt 2 had a severe OSA (AHI=78), while Pt 11 and 12 had no SRBD, but a markedly elevated PLMS index (respectively 66 and 80) which could justify why they could not reach SWS stages. One cervical SCI patient (Pt 2), and one thoracic SCI (Pt 8) did not present REM sleep throughout the night-time recording. As previously stated Pt 2 was obese with severe OSA, while Pt 8 was normoweight, had no SRBD and no PLM, but had a prolonged nap lasting 2 h and 21 min during the light-on period (around 11:00 am) in which presented 15 min of REM (data not shown).

The significant reduction in SWS we observed in tetraplegics and paraplegics could be due to several factors: SRBD may exert an important role in determining an increase proportion of light sleep stages due to the sleep fragmentation and the difficulty to reach deep sleep caused by the recurrent apneas. Therefore the higher incidence of OSA and the significantly increased number of arousals may explain the alteration in sleep architecture observed in cervical SCI. Although, SRBD could not explain why thoracic SCI presented a reduction in SWS similar to tetraplegic and singificantly different form controls, since thoracic SCI AHI was similar to controls. Therefore, as demonstrated by previous studies, the sleep disturbances observed in patients with SCI cannot be explained solely by disruptions in breathing during sleep [1,8,19].

Previous studies reported a high prevalence of PLMS in SCI patients [27-32], and suggested that PLMS causing frequent arousals may alter the sleep architecture. Although, in our study PLMS were present only in one cervical SCI (Pt 3, normoweight with severe OSA) and two thoracic SCI (Pt 11 and 12, see above), and were not strictly related to the AI.

A further possible explanation of the observed alteration in the sleep pattern in SCI patients involves melatonin [20]. Since the neural pathway for the endogenous production of melatonin passes through the cervical spinal cord [33,34] and patients with cervical SCI have an absence of the normal endogenous melatonin secretion at night [20,33] it was suggested that the alteration in night time melatonin secretion could chronically reduced sleep quality in cervical SCI patients [20]. Even if we believe that the disturbance in melatonin secretion may play a role in determining the disturbed sleep in cervical SCI patients, this hypothesis could not explain the sleep alteration we observed in paraplegics, whose melatonin secretion is supposed to be unaffected.

Therefore, other possible mechanisms may be involved. These mechanisms could be either the direct consequence of the injury or due to concomitant conditions or non specific factors.

Interestingly, thermoregulatory and hypnic mechanisms are thought to be linked [15]. Dependent on the site and extent of the lesion, individuals with a spinal cord injury (SCI) depend on the region above the lesion for thermoregulation [35,36] and may present a thermal dysfunction [35,37,38]. Therefore, it is tempting to speculate that the impaired thermoregulatory function in SCI patient may explain the sleep alteration we observed in cervical and thoracic SCI (see Section III). Although, the reverse could be also possible, being the reduced SWS to interfere with body temperature changes.

Further studies will be necessary to shed light on the possible pathogenetic mechanism of the reduction of SWS in cervical and thoracic SCI. Ideally, studies should be designed to investigate the effects of various interventions (e.g. treatment of OSA, PLMS or correction of thermal dysfunction) on normalization of sleep pattern in individuals with SCI.

Limitations

The main limitation of our study is that during V-PSG, due to the limitations deriving from the many complex issues related to SCI patients handling, we could record only a single respiratory channel and no SO_2. Therefore the occurrence of apneas during the V-PSG could not be assessed. Although, all subjects were questioned about SRBD disturbances during history taking and underwent a nocturnal cardio-respiratory monitoring (excluding Pt 1 and 4 and all controls). Furthermore, for ethical reasons we could not discontinue medications such as benzodiazepines and baclofen, which have a depressant effect on the central nervous system, increase the frequency and severity of SDB [39-41] but reduce PLMS [42].

Conclusions:

This study represent the largest V-PSG study in chronic SCI patients ever published and demonstrate a compromised sleep in patients with chronic cervical and thoracic SCI, characterized by an augmented proportion N2 NREM sleep and reduced SWS compared to controls. No statistical differences in any sleep parameter were found between cervical and thoracic SCI patients. While the sleep disturbances in cervical SCI could be related to a significantly higher AHI, and possibly to an alteration in melatonin secretion, the pathogenesis of the sleep disorder in thoracic SCI is jet to be determined.

Patient 10

Several case reports have described PLMS in relation to various spinal cord lesion, including traumatic SCI [27-32,43]. The temporal relationship between the lesion occurrence and the PLMS presentation, and the reduction of PLMS in relation to the amelioration of the clinical conditions suggested a causal relationship between the two events. Furthermore, occurrence of PLMS in SCI led to formulation of pathogenetic mechanisms pointing to a possible participation of the Central Pattern Generators (CPG) and the flexor afferent reflex in the physiopathology of PLMS as discussed by Paulus and Schomburg [43,44].

Interesting we had the chance to assess a patient (Pt 10) with a D8 Brown-Séquard syndrome. He was male, 47 years old, with a BMI=22,99. He was on Baclofen 50 mg/die and oxibutinin 30 mg/die.

Patient 10 was not included in the study (who was limited to complete Frankel A SCI patients) but underwent V-PSG, which demonstrated the occurrence of spontaneous leg movement during sleep on both legs, which did not fulfill the criteria for PLMS.

We believe that assessing by means of formal V-PSG further patients with Brown-Séquard syndromes may shed light on the intriguing physiopathology of PLMS.

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Section II

ANALYSIS OF CARDIOVASCULAR PARAMETERS

"Spinal cord injury is a ferocious assault on the body that leaves havoc in its wake. Paralysis is certainly part of its legacy, but there are other equally devastating consequences including autonomic dysfunction: compromised cardiovascular, bowel, bladder, and sexual function. Treatments and cures for these losses would greatly improve the quality of life for all of us living with spinal cord injury. I am hopeful that the multi-faceted and collaborative approach to spinal cord repair ... means that there will be effective therapies for autonomic dysfunction in the not too distant future."

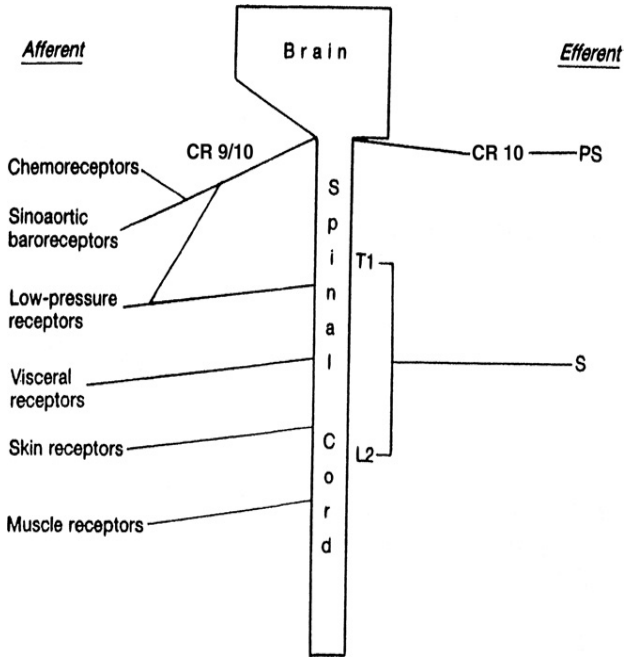
Christopher Reeve, September 30, 2004.

Section II.1: Autonomic control of the cardiovascular system during wakefulness

Background:

Normal functioning of the autonomic nervous system is critically dependent on integrity of the spinal cord, as the entire sympathetic outflow (T1-L2/3) and the sacral parasympathetic outflow travel and synapse within the spinal cord, before supplying various target organs (Fig II.1.1).

Fig II.1.1: schematic outline of the major autonomic pathways controlling the circulation



From Mathias C and Low D (2012) Autonomic Disturbances in Spinal Cord Injuries. In: Robertson D (ed) Primer on the autonomic nervous system. pp 505-509.

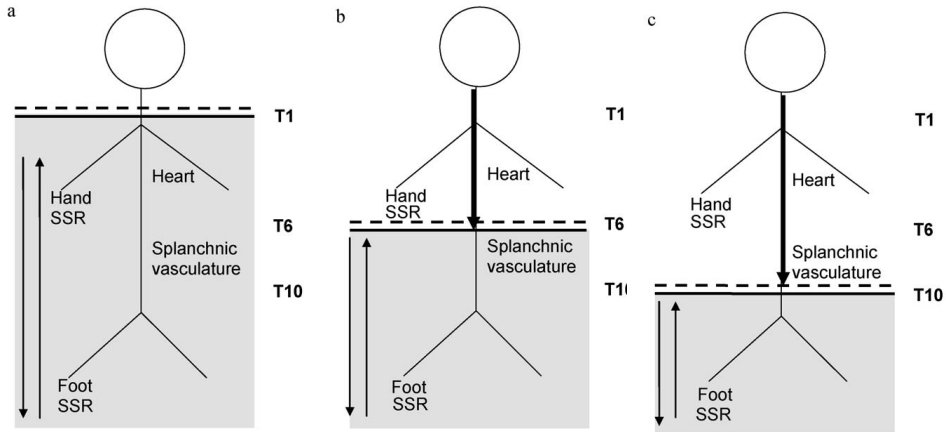
Schematic outline of the major autonomic pathways controlling the circulation. The major afferent input into the central nervous system is through the glossopharyngeal (CR,9) and vagus (CR,10) nerves by activation of baroreceptors in the carotid sinus and aortic arch. Chemoreceptors and low pressure receptors also influence the efferent outflow. The latter consists of the cranial parasympathetic (PS) outflow to the heart via the vagus nerves, and the sympathetic outflow from the thoracic and upper lumbar segments of the spinal cord. Activation of visceral, skin, and muscle receptors, in addition to cerebral stimulation influences the efferent outflow. In high spinal cord lesions, therefore, the input from chemoreceptors and baroreceptors is preserved along with the vagal efferent outflow, but there is no connection between the brain and the rest of the sympathetic outflow. The spinal sympathetic outflow may be activated through a range of afferents (visual, skin, muscle). This occurs through isolated spinal cord reflexes, not controlled by cerebral pathways, as seen normally.

Therefore spinal cord injury (SCI) results not only in paralysis; but it is also associated with a range of autonomic dysregulation that can interfere with cardiovascular, bladder, bowel, temperature, and sexual function. The entity of the autonomic dysfunction is related to the level and severity of injury to descending autonomic (sympathetic) pathways [1-3]. For the sake of the comprehension, we can consider three types of SCI lesions that offer typical autonomic reactions (tetraplegics, paraplegics at T6 and paraplegics at T10) (Fig II.1.2):

1. In cervical transection, the entire part of the sympathetic outflow, together with the sacral parasympathetic outflow, is totally disconnected from cerebral control (Fig II.1.2 a), causing an autonomic malfunction which may affect the cardiovascular, thermoregulatory, sudomotor, gastrointestinal, urinary and reproductive systems [4]. Conversely, all somatic and visceral afferents coming from below the lesion (starting from T1–T2 dermatomes) can reflexively activate the whole sympathetic column. Postural challenges cause an immediate fall in both systolic and diastolic blood pressure (SBP, DBP), due to the lack of rise in total peripheral resistance (TPR) [5,6], which is inversely related to a rapid rise in heart rate (HR), due to withdrawal of vagal tone in response to unloading of baroreceptor afferents [5-8]. Similar cardiovascular findings are observed during a static maximum inspiratory breath-hold [9] or the Valsalva manoeuvre (VM) [10]. After the VM, the blood pressure (BP) overshoot relative to baseline is absent [10]. Pressor stimuli that either originate in, or are modulated by, the brain (either cerebral or cervical afferents) will have no effect [11]. Pressor stimuli below the lesion will activate sympathetic reflex along the isolated spinal cord that can result in autonomic dysreflexia (AD).

2. In T6 paraplegics, there is a normal or near normal supraspinal control on the sympathetic innervation of the heart, lungs and upper limbs which originated from T1 to T5 (Fig II.1.2 b), while the T5–T10 segments, which controls the splanchnic vasculature and the lower limbs are isolated from cerebral control [4]. During postural challenges, there is usually an initial fall in BP, similar to tetraplegics, but followed by a more pronounced rise in HR that can partially compensate for the fall in BP [9,11]. During the VM, the BP initially decreases but then remains stable in paraplegics. However, at the end of the VM, the shortfall in BP relative to the baseline is similar in tetraplegics and paraplegics. After the VM, the BP overshoot relative to baseline is observed but significantly smaller in paraplegics than in controls [10]. Pressor stimuli above the lesion will elevate the HR, generally with a slight rise in BP [12,13] and skin vasoconstriction [14]. This response is not accompanied by changes below the lesion of the peripheral resistance of the leg [15], of electrodermal activity [16,17] or skin vasomotor reflexes [14]. Stimuli below the lesion may cause AD. However, the rise in BP is less severe in these patients than in tetraplegics [18-21].

Fig II.1.2: three types of spinal cord injury lesions that offer typical autonomic reactions



From Previnaire et al. (2010). Ann Phys and Rehab Med 53 (8):520-532.

Three patients presenting with complete somatic (ASIA Impairment Scale [AIS]A) lesions. a: tetraplegic patient with a sympathetic level of lesion above T1; b: T6 paraplegic patient with a sympathetic level of lesion at T6; c: T10 paraplegic patient with a sympathetic level of lesion at T10. Schematic representation of the cardiovascular and sudomotor sympathetic innervation, and the supraspinal control. Dotted horizontal line: ASIA somatic level of lesion; full horizontal line: sympathetic level of lesion; full vertical central arrow (when present): normal cardiovascular and sudomotor supraspinal control; grey shaded area: the isolated spinal cord and its extent (double vertical arrows).

3. In low paraplegics at (or below) T10, there is a normal or near normal supraspinal control on the entire sympathetic column (Fig II.1.2 c), with the exception of the T10–L2 sympathetic outflows, which is involved in sphincteric functions (lower urinary tract, anorectal and sexual functions). Depending upon the level of lesion, i.e. partial or total preservation of the sympathetic outflow to the splanchnic vessels and the legs, near normal or normal cardiovascular responses are expected during postural challenges. Stimuli above the lesion elicit a normal rise in BP and HR in this group. Near normal vasomotor reflexes on upper and lower limbs have been described following a variety of pressor stimuli [14]. These patients are less at risk of AD after stimuli below the lesion, though sporadic cases have been reported [22].

The sympathetic skin response (SSR) was proposed as useful tool to investigate autonomic involvement in SCI, and, in addition to motor and sensory evaluation, may improve classification of the extent of spinal functional deficits [23]. In cervical transection, following stimulation of the median or the supraorbital nerves, the SSR is

absent in the hands and feet [1,24-29]. In T6 paraplegics following stimulation of the median nerve, the SSR is absent in the feet (see discussion below). The palmar SSR can be elicited if the T4–T5 spinal cord segment is located above the lesion level [1,24-29]. In low paraplegics on median nerve stimulation, plantar SSR are rarely found in T10–T12 patients, but more consistently found with lesion below T12 [1,24,26,27,29].

Beside the severe impact on quality of life, cardiovascular dysfunction is the leading cause of morbidity and mortality in SCI individuals [30,31]. This most commonly manifests as neurogenic shock (in acute SCI) [3]; supine hypotension [3]; orthostatic hypotension (OH: marked decreases in blood pressure in the upright position) [1,32]; AD (profound and life-threatening blood pressure increases following afferent stimulation below the lesion) [3]; ECG abnormalities and arrhythmia [33], particularly during AD [25,34]; and abnormal cardiovascular responses to exercise [26]. Therefore laboratory evaluations to detect the presence of autonomic dysfunction and quantitate its severity are recommended.

Although assessment of autonomic function through standardized battery of clinical tests [35] is always preferable, it require specialized equipment, may be invasive, and, thus, may be difficult to perform routinely in clinical practice. The assessment of heart rate variability (HRV) instead, is a simple and reliable measurement of sympathetic and parasympathetic cardiac control. It is noninvasive, requires relatively short (10–15 minutes) recording periods in a rested state with no positional changes, and can be performed with easily accessible devices. Furthermore it is highly reproducible in persons with SCI [36].

HRV may be evaluated by a number of methods. Assessment of HRV in the frequency domain identifies three main spectral components: very low frequency (VLF), low frequency (LF), and high frequency (HF) components. High-frequency (HF, ~0.25 Hz) component of HRV represents cardiac vagal control [37-39]. LF HRV is more controversial but is generally accepted to be due to oscillations in vagal outflow generated through the baroreflex and driven by sympathetically induced LF blood pressure variability [37,40-42]. Therefore LF power is a measure of both sympathetic (mainly) and parasympathetic activities. Consequently LF/HF ratio is a measure of sympathovagal balance. Total Power (TP) is the sum of the three components and is considered a global measure of HRV.

Previous studies on HRV in SCI demonstrated that LF component was missing or significantly decreased in cervical SCI as compared to controls and patients with thoracic SCI in both supine and upright positions. Normalized HF power was significantly less in patients with thoracic SCI group as compared to cervical SCI in the supine position and tended to be greater in the cervical SCI group than in the thoracic SCI and control groups in the upright position [43]. LF/HF power ratio in cervical SCI compared to control and thoracic SCI groups was found to be either significantly larger [44] or lower [43] or even normal [7].

Objective:

Aim of our study was to assess the autonomic control of cardiovascular reflexes and HRV during supine rest (baseline) and head-up tilt test (HUTT) in chronic SCI patients.

Materials and Methods:

Subjects

Five cervical SCI with tetraplegia (C4-7), 7 thoracic SCI with paraplegia (D2-12) (Table II.1.1) and 26 age and sex matched control subjects (CNT) underwent assessment of cardiovascular reflexes and HRV while supine and tilted. Prior to data collection, all subjects underwent clinical examination. None of the subjects reported

symptoms or showed signs of diabetes, cardiorespiratory disease or of other pathological conditions that might affect autonomic cardiovascular control. The investigation conformed with the principles outlined in the Declaration of Helsinki [45]. The protocol was approved by the Institutional Review Board of the University of Bologna, and all participants provided informed consent.

Study protocol

All subjects were assessed in the morning, between 8 am and 12 am, in a quiet, temperature controlled clinical investigation room (23±1°C). Before the tests participants were allowed to have a light breakfast but had to abstain from smoking or drinking alcohol or caffeinated beverages from the night before the study. For ethical reasons, patients were allowed to use their usual medication, but all of them delayed their morning dose until after the experiment. Only one patient (Pt 12), who was on intrathecal baclofen and morphine could not postpone his treatment. Patients and controls were asked not to sleep or talk during the study.

All participants underwent HUTT, deep breathing (DB) and cold face (CF) under controlled laboratory conditions. Paraplegics and controls performed also VM and isometric exercise (IE) test.

SBP and DBP (Portapres model 2, TNO-TPD Biomedical Instrumentation), heart rate (Grass 7P511 and Light Work Station for digital RR quantification), oronasal and abdominal breathing (Grass DC preamplifier 7P1) were monitored continuously. Finger pressure detected by Portapres can be measured between 10 and 300 mmHg and the internal accuracy of the instrument is 1.5% of full scale.

After 30 min of supine rest, the HUTT (10 min at 65°), VM (40 mmHg for 15 sec), DB (6 breaths/min), CF test (60 sec of application of cold compresses at 0-1°C to the forehead), and IE (30% of maximal effort for 5 min) were performed using standard procedures [35]. The correct execution of tests was checked automatically by an electronic device which displays time and execution of the manoeuvre. Tests were repeated until they were performed with an error < 5% with respect to the expected procedure. The manoeuvres were carried out in the sequence described, allowing a period of rest to reach basal BP and HR values in between investigations. The results of each test were automatically obtained by means of home-developed software. The mean values of SBP, DBP and HR during the last minute of HUTT were compared to the baseline (obtained calculating the mean value of the last five minutes of supine rest preceding HUTT). During the Valsalva manoeuvre, the following indices of autonomic activity were considered: the ratio between HR in phases II and IV (VR) and the overshoot during phase IV (OV=difference between the highest SBP after the expiratory effort and the basal value). During deep breathing, the sinus arrhythmia (calculated in beats per minute using the ten longest R-R intervals during expiration and the ten shortest R-R intervals during inspiration) and the inspiratory-expiratory ratio (I/E=ratio between the mean of higher HR values during ten deep inspirations and the mean of the lower HR values during expirations) were calculated. During the cold face test, the changes (Δ) with respect to the basal value of SBP, DBP and HR after 60 sec of application of cold compresses (0-1°C) to the forehead were computed. During sustained handgrip, the changes with respect to the basal value of SBP, DBP and HR were calculated after 5 min of isometric effort.

Spectral analysis of heart rate variability

Autoregressive power spectral analysis (PSA) of HRV during supine rest and HUTT was performed using the dedicated HRV Analysis Software 1.1 for Windows developed by the Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland (http:// venda.uku.fi/research/biosignal) [46] according to standard procedures [47]. We considered a high-frequency (HF), respiratory-linked component

(centered to ~0.25 Hz, reflecting mostly vagal activity), and a low frequency (LF) component (centered at ~0.1 Hz, reflecting mostly sympathetic activity). The oscillatory components between 0 and 0.03 Hz were considered VLF. Each spectral component is presented in normalized form, obtained by dividing it by the total power less the VLF, if present, and multiplied by 100. The LF/HF ratio was used as an index of sympathovagal balance. We also calculated the total power spectrum (ms2) resulting from the sum of VLF power, LF power and HF power in absolute values. Owing to its inherent nonlinearity, the signal from the nasal thermistor was used only to assess the main respiratory frequency of the period considered for the evaluation of HR variability. The power spectral component centered at ~0.25 Hz was considered HF only if coincident with the main respiratory frequency.

Table II.1.1: patients clinical characteristics and medical therapy

Pt n°	Group	Lesion Site	Dis Hx (yrs)	Age (yrs)	BMI	OH symptoms	Therapy / day
1	C4-7	C4	15	32	24	YES	- baclofen 100 mg - tamsulosin hydrochloride 0,4 mg
2	C4-7	C4-C5	11	44	28	YES	- risperidone 2mg - bisacodyl 10 mg - triazolam 125 mcg - lactulose
3	C4-7	C5-C6	25	60	23	YES	- dantrolene sodium 75 mg - valproic acid 500 mg - lorazepam 2,5 mg - baclofen 75 mg - terazosin 5 mg
4	C4-7	C5-C6	2	16	23	YES	- sertraline hydrochloride 50 mg
5	C4-7	C6-C7	1	54	22	YES	- dihydroergotamine 2mg/ml 15 - tamsulosin hydrochl 0,4 mg - desmopressin 60 mcg - dalteparin sodium 5000 U - gabapentin 600 mg - baclofen 50 mg - oxybutynin 10 mg - citalopram 20 mg - lansoprazole 15 mg
6	D2-12	D2-D3	4	24	18	NO	- baclofen 75 mg - pregabalin 300 mg - trospium chloride 40 mg - lansoprazole 30 mg - clomipramine 25 mg
7	D2-12	D4	1	59	29	NO	- hydroclorotiazide 25 mg - carvedilol 12,5 mg - amlodipine 5 mg - ramipril 5 mg - amiloride 2,85 mg - citalopram 10 mg
8	D2-12	D4-D5	1	34	21	NO	- baclofen 75 mg - oxybutynin 15 mg - pregabalin 150 mg - tizanidine 3 mg
9	D2-12	D7-D8	0,5	26	23	NO	- oxybutynin 10 mg - etidronic acid 1200 mg
11	D2-12	D9	1	61	23	NO	- triazolam 125 mcg - paroxetine 20 mg - enoxaparin sodium 6000 U - oxybutynin 15 mg - diltiazem 30 mg - lansoprazole 30 mg - tizanidine 18 mg
12	D2-12	D10	3	31	31	NO	- intrathecal baclofen 0,5 mg - intrathecal morphine 1,5 mg
13	D2-12	D12	1,5	44	30	NO	– nil

BMI=body mass index; Dis Hx=disease history; OH symptoms=symptoms of orthostatic hypotension; Pt n°=patient number; yrs=years.

Statistical analysis

Data are reported as means ± SD. Between subjects data, which were normally distributed, were compared using a repeated measures analysis of variance (ANOVA) with Bonferroni and Dunnett's T3 post hoc tests for multiple comparisons as appropriate. Between subjects data from power spectral analysis of HRV, expressed as normalized unit and not normally distributed, were analyzed using the Kruskal–Wallis test. Within subjects data were compared by means of related samples repeated measure t-test or by Wilcoxon signed-ranks test for related samples depending on if they were normally distributed or not. All statistical analyses were performed with IBM SPSS Statistics version 20.0 and a $p<0,05$ was considered significant.

Results:

Subjects characteristics

Patients characteristics and medical therapy are listed in Table II.1.1. All subjects were males. Cervical SCI ranged from C4 to C7. Thoracic SCI ranged from T2 to T12. Mean age±SD was 41±18 years for tetraplegics, 41±15 years for paraplegics and 46±11 years for controls. All patients had a complete Frankel A spinal cord lesion, and they were clinically stable (the traumatic event occurred more than 6 months before the time of the study). All SCI patients had a traumatic lesion, with the exception of patient 7 who suffered a spinal cord ischemic lesion. Cervical SCI had a disease history of 11±10 years (min-max=1-25), while in thoracic SCI ranged from 0,5 to 5 years with a mean of 2±1 years. The control group was composed by age matched drug free male volunteers. There were no age or BMI differences between groups (Table II.1.2). All tetraplegics but no paraplegics nor controls reported symptoms of OH (Table II.1.1).

Table II.1.2: age and Body Mass Index (BMI) in cervical and thoracic SCI patients and controls

Parameter	CNT (n= 24)	C4-7 (n= 5)	D2-12 (n= 7)
Age (years)	40±15	41±18	40 ±15
BMI	25,4±2,9	23,8±2,5	24,8±5

Values are means ± SD.

Sympathetic skin response (SSR)

All patient underwent SSR testing before enrollment to assess neurogenic activation of sweat glands (Table II. 1.3). As expected all cervical SCI had absent SSR in all limbs. Thoracic SCI patients 7 to 12 had absent SSR in lower limbs and normal SSR in upper limbs. Patient 6 had absent SSR in all limbs, while patient 13 had normal SSR in all limbs and sacral region.

Table II.1.3: patients clinical characteristics and SSR

Pt n°	Group	Lesion Site	Age (yrs)	BMI	SSR
1	C4-7	C4	32	24	absent in all limbs
2	C4-7	C4-C5	44	28	absent in all limbs
3	C4-7	C5-C6	60	23	absent in all limbs
4	C4-7	C5-C6	16	23	absent in all limbs
5	C4-7	C6-C7	54	22	absent in all limbs
6	D2-12	D2-D3	24	18	absent in all limbs
7	D2-12	D4	59	29	normal UULL (LH<RH), absent in LLLL
8	D2-12	D4-D5	34	21	normal UULL , absent in LLLL
9	D2-12	D7-D8	26	23	normal in UULL, absent in RF, partial in LF
11	D2-12	D9	61	23	normal UULL, absent in LLLL
12	D2-12	D10	31	31	normal UULL, absent in LLLL
13	D2-12	D12	44	30	normal in all limbs and sacral region

BMI=body mass index; LF=left foot; LH=left hand; LLLL=lower limbs; Pt n°=patient number; RF=right foot; RH=right hand; SSR= sympathetic skin responses; UULL=upper limbs; yrs=years.

Head Up Tilt Test (HUTT)

All patients but one (Pt 2) underwent a 10 min 65° HUTT. During the orthostatic challenge the tilt angle was adjusted to 30° in Pt 4 and to 50° in Pt 5, due to the occurrence of OH symptoms, but they were all able to complete the test following this adjustments.

While resting supine, tetraplegics compared to controls presented significantly lower values of SBP and DBP and HR (p=0,035, p=0,029 and p=0,013 respectively) (Table II.1.4; Fig II.1.4). Tetraplegics compared to paraplegics presented a significantly lower baseline HR (p=0,004) (Table II.1.4; Fig II.1.4). No significative differences were observed in baseline supine SBP, DBP and HR between paraplegics and control subjects (Table II.1.4; Fig II.1.4).

During HUTT, SBP and DBP values were significantly lower in tetraplegics compared to paraplegics and controls (SBP: C4-7 *vs* CNT and C4-7 *vs* D2-12 p<0,001 on both comparisons; DBP: C4-7 *vs* CNT p<0,001; C4-7 *vs* D2-12 p=0,047) (Table II.1.4; Fig II.1.4). HR values during HUTT were significantly lower in cervical SCI compared to thoracic SCI (p=0,03), but no differences were observed between tetraplegics and controls (Table II.1.4; Fig II.1.4). All four (Pt 1, 3, 4 and 5) tetraplegics who performed the orthostatic challenge had a BP fall which fulfilled the criteria for OH [48], and two of them reported symptoms of orthostatic intolerance (Pt 4 and 5) (Fig II.1.3). Only one paraplegic (Pt 8) presented a similar fall, which was asymptomatic (Fig II.1.3). None of the controls had OH during HUTT (Fig II.1.3).

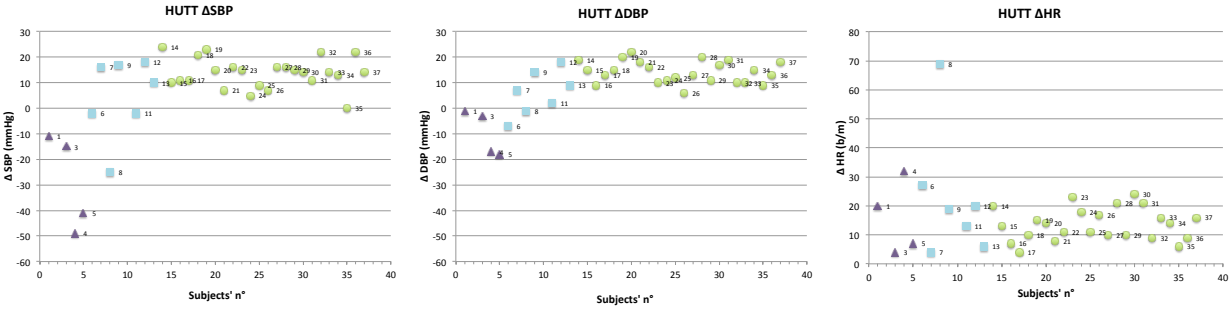
During the orthostatic challenge controls presented a statistically significant increase in SBP, DBP and HR compared to their baseline values (p<0,001 in all comparisons), while no significant within group changes were observed in tetraplegics (Table II.1.3; Fig II.1.4). Paraplegics during HUTT demonstrated a significant increase in HR (p=0,035) compared to baseline values (Table II.1.3; Fig II.1.4).

Table II.1.4 : SBP, DBP and HR values while supine and during HUT in SCI patients and controls

Condition	Parameter	CNT (n=24)	C4-7 (n=4)	D2-12 (n=7)
baseline	**SBP (mmHg)**	119±10*	102±13	119±22
	DBP (mmHg)	64±7*	56±5	61±15
	HR (b/m)	62±7*	51±3	65±9*
HUTT	**SBP (mmHg)**	133±10*,°	75±14	124±32*
	DBP (mmHg)	79±9*,°	47±11	67±15*
	HR (b/m)	75±9°	67±13	88±20*,°

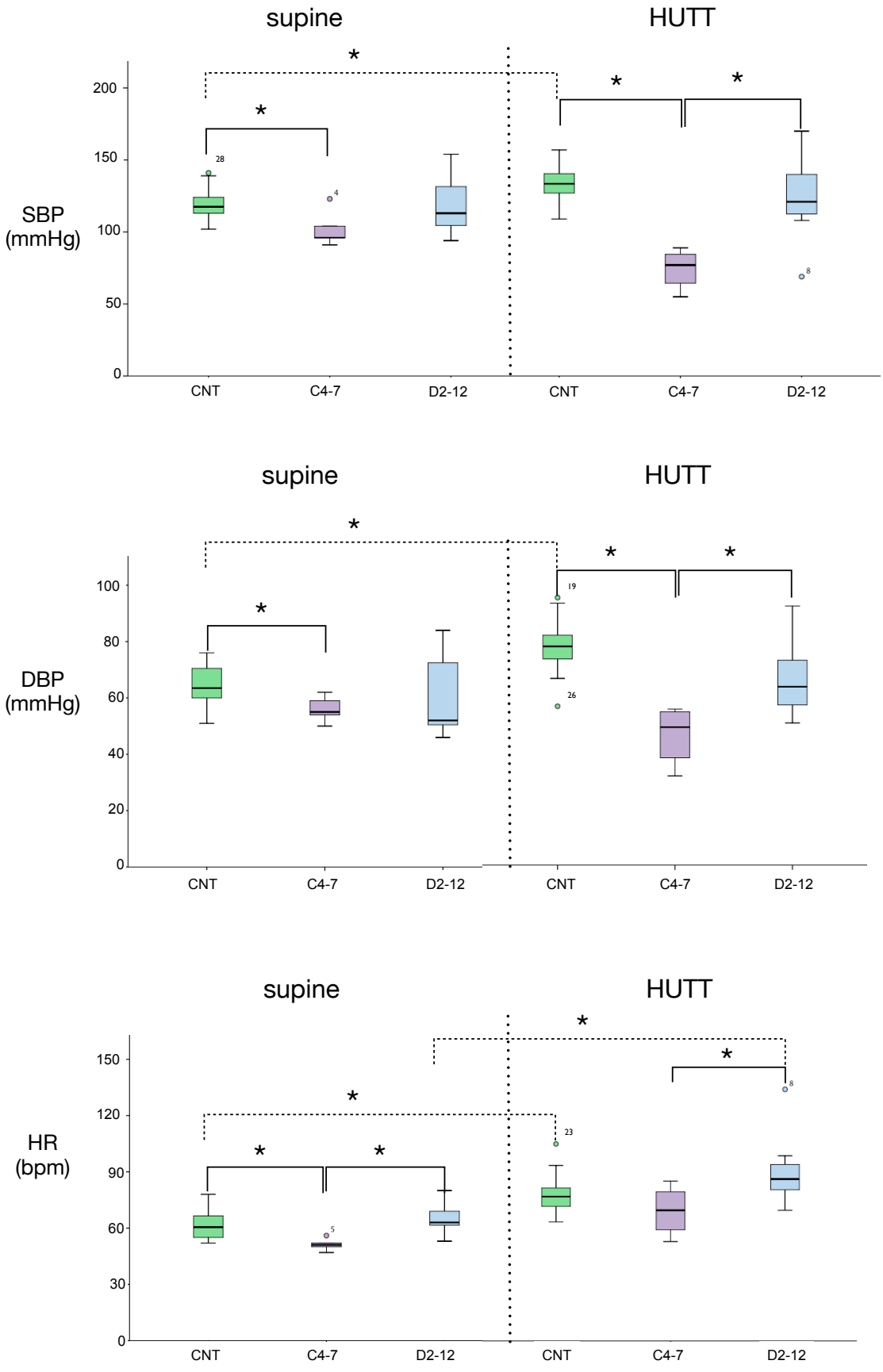
Values are means ± SD. *=p<0,05 *vs* cervical SCI. °=p<0,05 *vs* baseline supine.

Fig II.1.3: changes (Δ) in SBP, DBP and HR during HUTT in SCI patients and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

Fig II.1.4: SBP, DBP and HR means±SD during supine rest and HUTT in SCI patients and controls

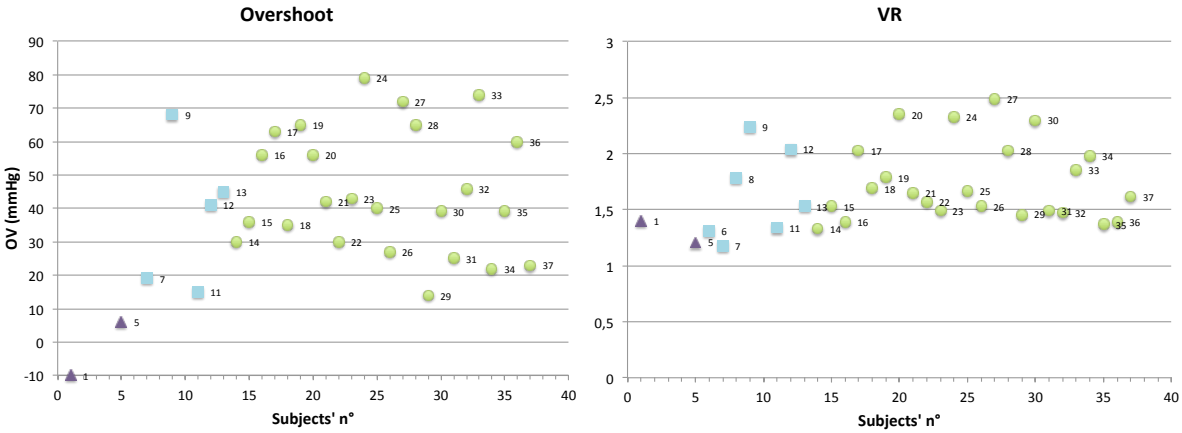


*=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI.

Valsalva manoeuvre (VM)

Only two (Pt 1 and 5) out of five tetraplegics were able to perform an adequate VM, while all paraplegics and controls could perform the manoeuvre without difficulties (Fig II.1.5). Due to this limitation we did not consider the data from the cervical SCI for statistical analysis. Although, what was evident was that the 2 cervical SCI (Pt 1 and 5), who performed the manoeuvre properly, had no overshoot (Fig II.1.5). Similarly, 2 thoracic SCI (Pt 6 and 8) had no overshoot after the VM, while the remaining 5 paraplegics and all the control subjects obtained normal results. No statistically significant differences were observed in overshoot and Valsalva ratio between paraplegics and controls (Table II.1.5).

Fig II.1.5: overshoot (OV) and Valsalva ratio (VR) in SCI patients and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

Table II.1.5 : Overshoot and Valsalva Ratio in SCI patients and controls

Parameter	CNT (n=24)	C4-7 (n=2)	D2-12 (n=7)
Overshoot (mmHg)	45±18	-2±11†	22±33
Valsalva Ratio (VR)	1,74±0,35	1,31±0,13†	1,63±0,4

Values are means ± SD.†=data not considered for statistical analysis due to the reduced sample size

Isometric exercise (IE)

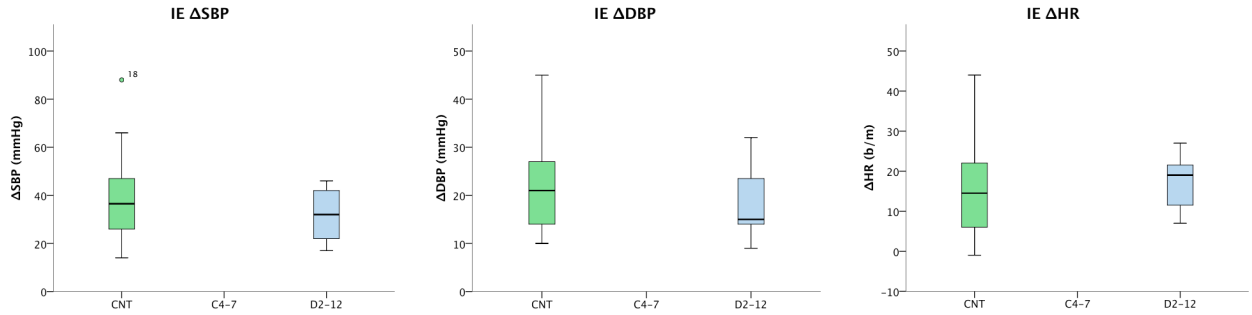
Tetraplegics could not perform IE. No significative differences were observed in SBP, DBP and HR between paraplegics and control subjects during supine rest and IE (Table II.1.6; Fig II.1.6; Fig II.1.7). During the sustained handgrip, compared to baseline, a significant increase in SBP, DBP and HR was observed both groups (p<0,001 in all comparisons) (Table II.1.6).

Table II.1.6: SBP, DBP and HR values at rest and during isometric exercise (IE) in SCI patients and CNT

Condition	Parameter	CNT (n=24)	C4-7 (n=0)	D2-12 (n=7)
baseline	**SBP (mmHg)**	127±14	NA	133±20
	DBP (mmHg)	72±8	NA	69±16
	HR (b/m)	63±12	NA	68±13
IE	**SBP (mmHg)**	166±24°	NA	165±24°
	DBP (mmHg)	94±15°	NA	88±14°
	HR (b/m)	78±12°	NA	85±10°

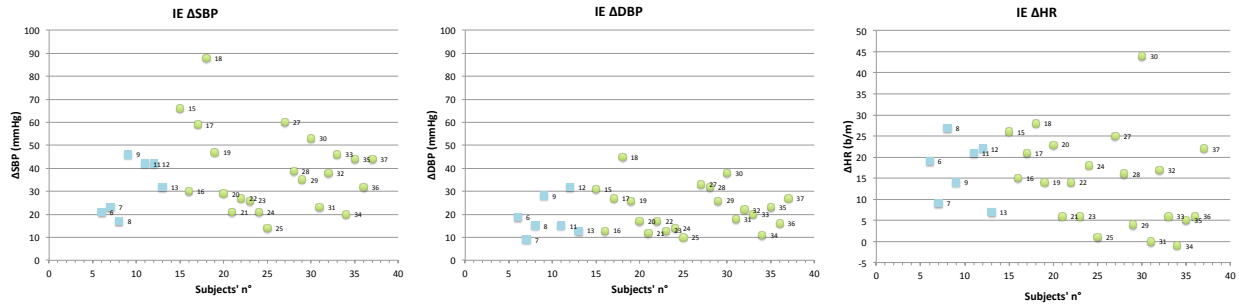
Values are means ± SD. °=p<0,001 *vs* baseline. NA: not applicable.

Fig II.1.6: mean ± SD changes (Δ) in SBP, DBP and HR during IE in SCI and controls



C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI.

Fig II.1.7: changes (Δ) in SBP, DBP and HR during Isometric Exercise (IE) in SCI and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

Deep breathing (DB)

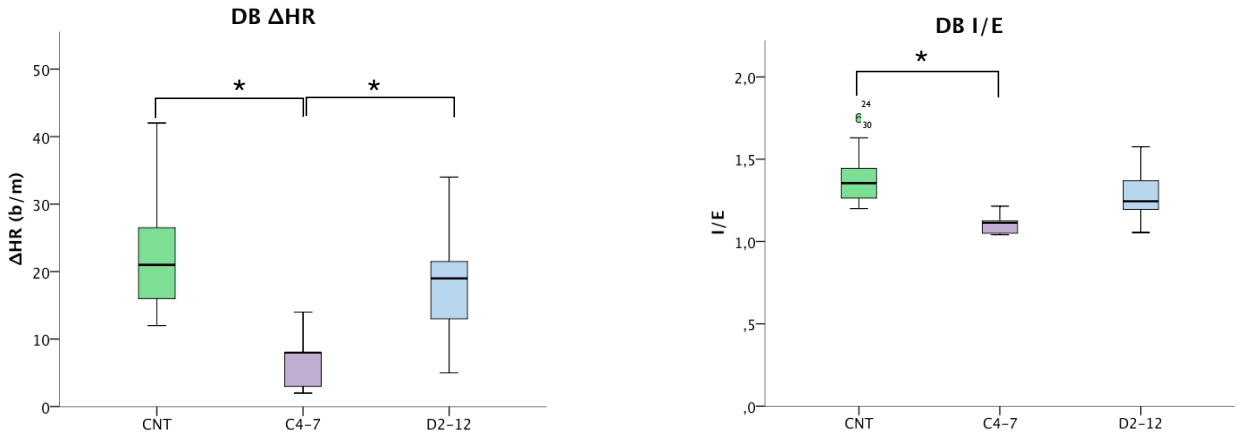
While resting supine on spontaneous breathing, tetraplegics, compared to controls, presented significantly lower values of HR (p<0,001) (Table II.1.7). During deep breathing, paced at 6 breaths/min, cervical SCI compared to controls and thoracic SCI presented a significantly lower respiratory arrhythmia (p=0,001; p=0,046 respectively) (Table II.1.7; Fig II.1.8; Fig II.1.9). The I/E was significantly lower in tetraplegics compared to controls (p=0,002) (Table II.1.7; Fig II.1.8; Fig II.1.9). No statistically significant differences in I/E were observed between cervical and thoracic SCI (Table II.1.7; Fig II.1.8; Fig II.1.9). All groups within themselves demonstrated a significant difference between inspiratory and expiratory HR (CNT: p<0,001; C4-7: p=0,031; D2-12: p=0,002) (Table II.1.7).

Table II.1.7: HR values at rest and during deep breathing (DB) in SCI patients and controls

Parameter	Condition	CNT (n=24)	C4-7 (n=5)	D2-12 (n=7)
HR	**Baseline**	65±10*	53±2	67±14
	Inspiration	79±10	69±12	86±10*
	Expiration	57±8§,°	62±8°	68±13°
	Δ Insp-Esp	22±7*	7±5	18±9*
	I/E ratio	1,4±0,2*	1,1±0,1	1,3±0,2

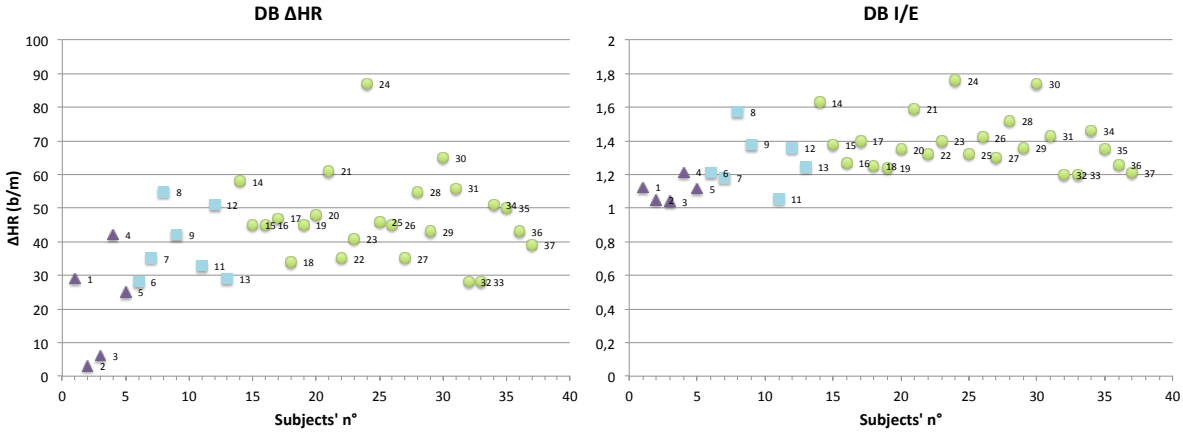
Values are means ± SD. *=p<0,05 *vs* cervical SCI. §=p<0,05 *vs* thoracic SCI. °=p<0,05 *vs* inspiration

Fig II.1.8: mean ± SD changes (Δ) in HR and I/E ratio during DB in SCI and controls



*=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; I/E: inspiratory/expiratory ratio.

Fig II.1.9: changes (Δ) in HR and I/E ratio during Deep Breathing (DB) in SCI and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; I/E: inspiratory/expiratory ratio. Numbers on axis of abscissas correspond to subjects' numbers.

Cold Face (CF)

Baseline SBP, DBP and HR was significantly lower in cervical SCI compared to controls (*p*=0,005; *p*=0,049; *p*=0,007 respectively) (Table II.1.8). Baseline SBP was significantly lower in tetraplegics compared to paraplegics (*p*=0,024) but no differences were observed in baseline DBP and HR between cervical and thoracic SCI (Table II. 1.8).

Cervical SCI compared to thoracic SCI and controls presented a significantly lower SBP, DBP values during the cold face test (SBP: C4-7 *vs* CNT *p*=0,001; C4-7 *vs* D2-12: *p*=0,003; DBP: C4-7 *vs* CNT *p*=0,002; C4-7 *vs* D2-12 *p*=0,042) and consequently demonstrated a significantly lower increase in SBP (ΔSBP) and DBP (ΔDBP) during the manoeuvre (ΔSBP: C4-7 *vs* CNT *p*=0,005; C4-7 *vs* D2-12: *p*=0,004; ΔDBP: C4-7 *vs* CNT *p*=0,019; C4-7 *vs* D2-12 *p*=0,012) (Table II.1.8; Fig II.1.10; Fig II.1.11). The mean HR was not significantly different in the three groups during the CF, but cervical SCI compared to controls presented a reduced decrease in HR (ΔHR) during the cold stimulation of the forehead (*p*=0,014) (Table II.1.8; Fig II.1.10; Fig II.1.11). No differences were observed in ΔHR between cervical and thoracic SCI (Table II.1.8; Fig II.1.10; Fig II.1.11).

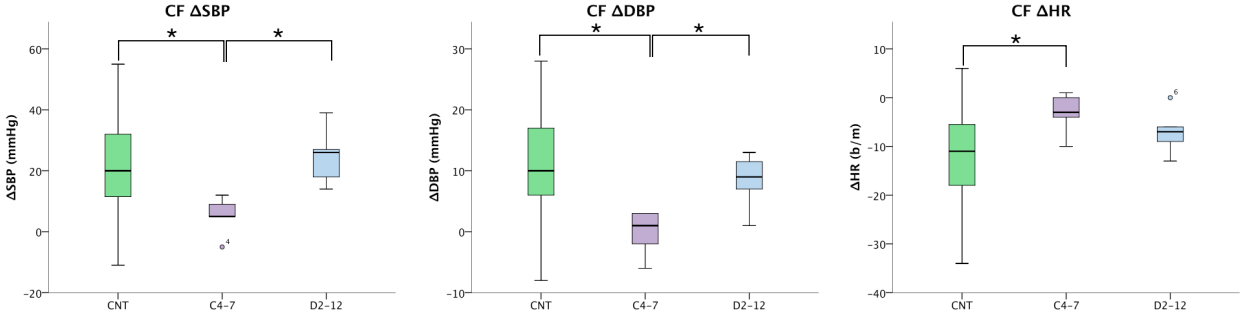
Within subjects analysis demonstrated that both thoracic SCI and controls presented significant increase in BP and decrease in HR while cervical SCI did not (Table II.1.8).

Table II.1.8: SBP, DBP and HR values at rest and during cold face test (CF) in SCI patients and CNT

Condition	Parameter	CNT (n=23)	C4-7 (n=5)	D2-12 (n=7)
baseline	**SBP (mmHg)**	132±15*	102±23	131±21*
	DBP (mmHg)	74±8*	61±10	72±17
	HR (b/m)	63±9*	53±4	68±16
CF	**SBP (mmHg)**	153±24*,°	108±18	155±22*,°
	DBP (mmHg)	85±14*,°	60±7	81±16*,°
	HR (b/m)	50±12 °	50±5	60±15 °

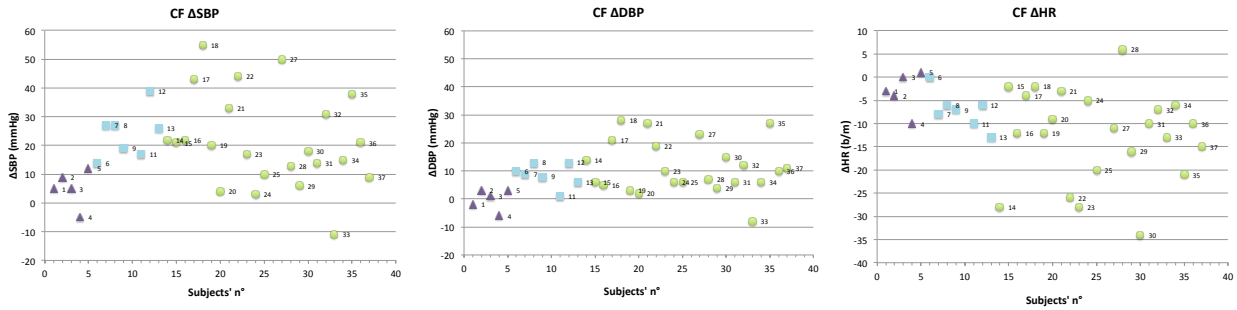
Values are means ± SD. *=p<0,05 *vs* cervical SCI. °=p<0,05 *vs* baseline.

Fig II.1.10: mean ± SD changes (Δ) in SBP, DBP and HR during Cold Face (CF) in SCI and controls



*=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI.

Fig II.1.11: changes (Δ) in SBP, DBP and HR during Cold Face (CF) in SCI and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

Analysis of heart rate variability

Data of autoregressive PSA are summarized in Table II.1.9. Respiratory frequency while resting supine was comparable in the three groups, while during HUTT it was significantly higher in controls compared to thoracic SCI patients (p=0,045).

Total power in HRV spectrum was similar in the three groups at rest and during HUTT.

During supine rest, there were no significative difference in the LF (nu) and HF (nu) components, and consequently in LF/HF, between the three groups. During HUTT, cervical SCI had a significantly lower LF (nu) (p=0,009) and a significantly higher HF (nu) (p=0,031), and consequently a significantly different LF/HF (p=0,019) compared to controls. No significant differences were observed between cervical and thoracic SCI during HUTT.

During HUTT, while the expected changes in PSA components were observed in controls (significant increase in the LF (p<0,001) and significant reduction of the HF (p<0,001)), these modifications were not detected in cervical and thoracic SCI patients who showed values of LF (nu) and HF (nu) during HUTT comparable to supine rest.

Table II.1.9: autoregressive PSA of HRV during supine rest and HUTT in SCI patients and CNT

Condition	Parameter	CNT (n=24)	C4-7 (n=5)	D2-12 (n=7)
Supine	**Respiratory frequency (Hz)**	0,26±0,05	0,24±0,07	0,21±0,08
	Total power spectrum (ms²)	1.466.401±1.638.496	1.243.542±991.335	1.069.240±604.458
	LF (nu)	55±17	47±23	65±24
	HF (nu)	35±15	45±23	21±19
	LF/HF	2,4±2,5	1,9±2,1	5,6±3,9
HUTT	**Respiratory frequency (Hz)**	0,30±0,06§,°	0,24±0,08	0,23±0,07
	Total power spectrum (ms²)	1.850.908±4.280.200°	2.730.180±4.449.798	608.017±604.426
	LF (nu)	80±14*,°	50±23	74±10
	HF (nu)	16±14*,°	40±6	20±12
	LF/HF	9,6±8,2*,°	1,7±1,3	4,8±2,6

Values are means ± SD. *=p<0,05 *vs* cervical SCI.§=p<0,05 *vs* thoracic SCI. °=p<0,05 *vs* baseline.

Discussion:

We investigated the autonomic control of cardiovascular reflexes and the PSA of HRV in 5 tetraplegics patients, 7 paraplegics SCI patients, and 24 healthy control subjects under controlled conditions.

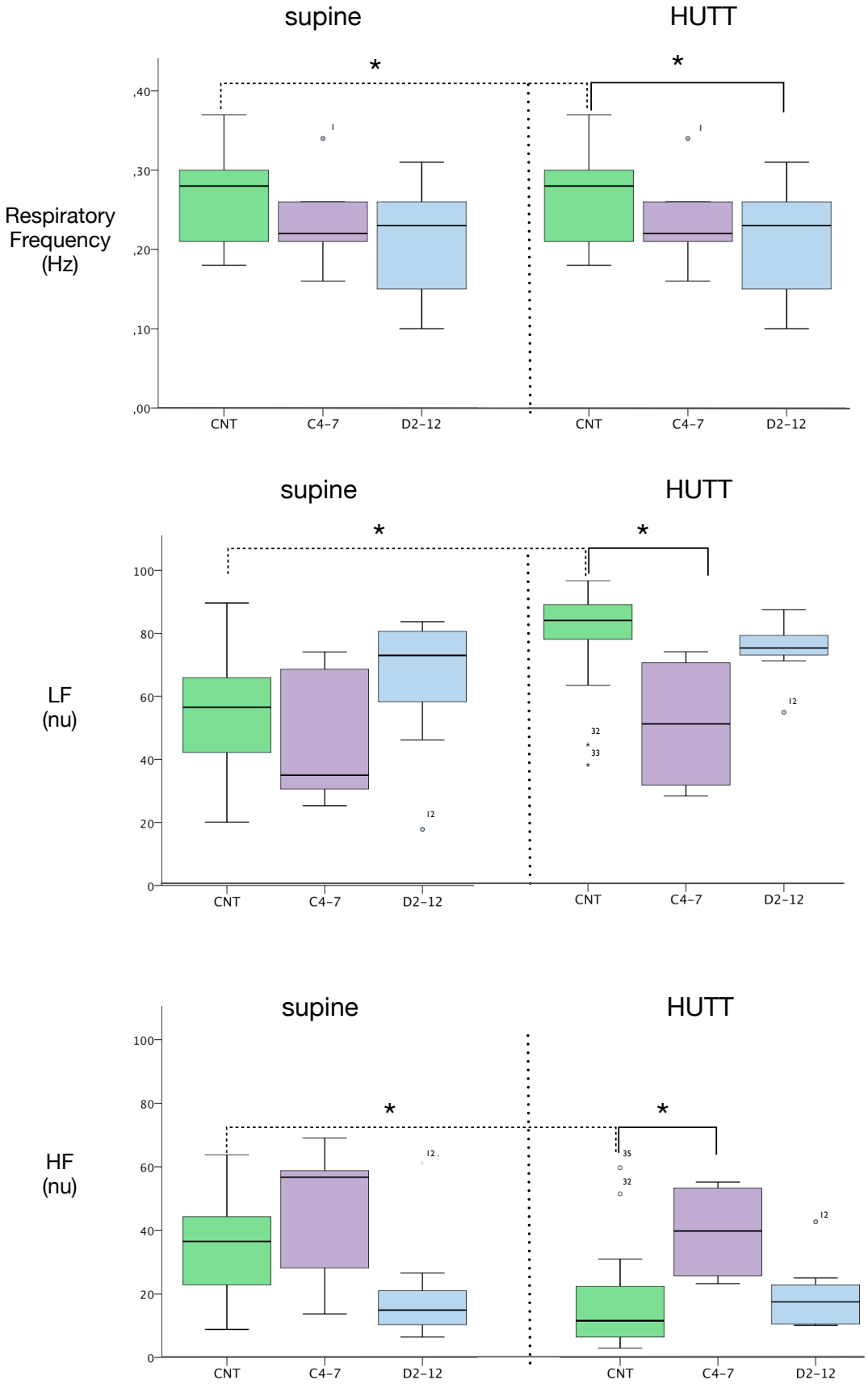
SSR

Palmar and plantar SSR are thought to be generated at the T1–T3 and T10–L2 sympathetic segments, respectively [24,29,49,50]. Absence of palmar and plantar SSR in all our tetraplegic patients indicates a total interruption of cholinergic pathways above T1. In paraplegics, absence of plantar SSR is indicative of an interruption at or above T10. The further absence of palmar SSR in one (Pt 6) T2-3 paraplegic might reflect more extensive sympathetic damage to the thoracic segment. In contrast, the presence of plantar SSR in patient 13 with T12 lesions indicates partially preserved sympathetic pathways for a few segments below the lesion, sufficient to produce plantar SSR [24].

HUTT

Our results confirm previous findings demonstrating lower resting BP in subjects with cervical SCI than controls [11,25,51,52]. Supine BP in thoracic SCI subjects and controls was similar. There is a known inverse linear relationship between level of SCI and resting BP [11,21], that is probably related to the associated reduction in basal sympathetic activity, and subsequent low plasma NA and adrenaline levels [11,53]. When upright, these BP differences were even more marked. Cervical SCI had a significantly lower SBP and DBP compared to controls and paraplegics. All four cervical SCI who underwent the orthostatic challenge had OH, while one out of seven paraplegic patients had a BP fall which fulfilled the criteria for OH. Only two cervical SCI reported symptoms of OH, confirming previous observations that despite marked OH, many SCI individuals are asymptomatic [26,54].

Fig II.1.11: respiratory frequency, LF (nu) and HF (nu) during supine rest and HUTT in SCI and controls



*=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI.

HR responses to orthostatic stress are due to baroreflex-mediated parasympathetic (vagal) withdrawal, usually preserved following SCI [21], and sympathetic activation. Lesions above T6 may disrupt descending sympathetic control of the heart. Resting supine HR in our cervical SCI patients was significantly slower compared to thoracic SCI and controls. Tetraplegics compared to paraplegics and controls did not present a significant increase in HR during the orthostatic challenge and therefore HR in cervical SCI, was significantly slower during HUTT compared to thoracic SCI. The alterations in cervical SCI resting HR have been seen previously [11,55,56], and probably reflect decreased sympathetic chronotropic responses in high lesions [56], and compensation for reduced SV in thoracic SCI [55]. Upright HR responses were similar between cervical SCI and controls, suggesting they are predominantly due to baroreflex-mediated reductions in vagal tone, which is preserved in all subjects. However, the stimulus provided to the baroreceptors would have been greater in cervical SCI subjects, in whom upright blood pressures were lowest. Accordingly, one might expect larger HR increases during orthostatic stress in cervical SCI subjects. The fact that this was not observed in our court may be partly due to increased cardiac baroreflex delay in cervical SCI subjects [1].

Although there were no significative differences between thoracic SCI and controls, within subjects analysis demonstrated that paraplegics, as tetraplegics, did not present a significant increase in HR. Therefore, even if cardiac autonomic control is intact in thoracic SCI, they had a blunted sympathetic response to HUTT [8].

Valsalva manoeuvre

VM induced no overshoot in the two cervical SCI patients who were able to perform it adequately, but it was present in most of paraplegics. These findings confirm that the OV is primarily related to persistent cardiac sympathetic activity [10]. Only two thoracic SCI (Patient 6 and 8 with T2-3 and T4-5 lesion respectively) showed no overshoot after the VM. Noteworthy patient 6 had absent SSR in all limbs and therefore a more extensive sympathetic damage.

Isometric exercise

Isometric exercise could be performed only by thoracic SCI, who demonstrated normal pressor responses compared to controls, probably due to an increase in sympathetic activity above the lesion level and an increase in heart rate and cardiac contractility [57]. Although, the rise is SBP was blunted as the splanchnic and lower limb vascular beds could not contribute to the hemodynamic response [12] as demonstrated by the absence of changes of the peripheral resistance of the leg [15], of electrodermal activity [16,17] or skin vasomotor reflexes [14].

Deep Breathing

During deep breathing cervical SCI compared to controls and thoracic SCI presented a significantly lower respiratory arrhythmia and the I/E was significantly lower in tetraplegics compared to controls. These finding might be due to inability to reduce vagal activity through the pulmonary inflation reflex because of the inability to breathe or an increased vagal activity in relation to hypoxia [58]. Although all groups, including tetraplegics, within themselves demonstrated a significant increase in HR during inspiration compared to expiration.

Cold Face

Stimulation of the forehead with ice-cold water induced no changes in BP or HR in cervical SCI. Consequently tetraplegic presented significantly lower SBP, DBP values during the manoeuvre compared to thoracic SCI and controls. Instead, no significative differences were observed between thoracic SCI and controls. These results are in agreement with previous findings demonstrating that stimuli above the lesion site have no effect on BP in tetraplegics [11]. Corbett et al. found no change in HR and BP following cutaneous (pinprick and forehead cold) or cerebral (mental arithmetic or noise) stimuli above the lesion [59]. Stimulation above the lesion is not accompanied by changes of the peripheral resistance of the leg or vasoconstriction in the calf [15,59], of electrodermal activity [16,17,24,50], or of skin vasomotor reflexes [14].

Tetraplegics during cold face had no bradycardia compared to paraplegics and controls. This finding might be due to the impossibility to elicit chronotropic responses due to disruption of sympathetic descending control to the heart [56].

Heart rate variability analysis

In our court the LF (nu) component of HRV was present and not significantly different in the three groups during supine rest. During HUTT cervical SCI, compared to thoracic SCI and controls, did not present and increase in LF (nu) and consequently demonstrated a reduced LF (nu) during the orthostatic challenge. Similarly HF (nu) was not significantly different in the three groups while supine, but did not decrease during the postural change and was significantly higher in tetraplegics compared to paraplegics and controls during HUTT. Consequently the LF/ HF was similar in the tree groups during supine rest, but was significantly lower in cervical SCI compared to thoracic SCI and controls during HUTT.

Inoue et al. [44] in a study on six completely quadriplegic patients found that LF component was missing in quadriplegics, indicating that sympathetic fibers integrity is required for LF component. Although Claydon and Krassioukov [43] demonstrated that the LF oscillations were not abolished in 14 patients with cervical SCI , but were reduced compared to 17 controls and 12 patients with thoracic cord injury, in both supine and upright positions suggesting that (1) LF after cervical SCI is mediated also by parasympathetic mechanisms, (2) spontaneous rhythmic firing of spinal sympathetic neurons, and (3) an incomplete destruction of descending sympathetic pathways [43,60].

Our results confirm previous findings by Claydon and Krassioukov [43] who showed that HF (nu) tended to be greater in cervical SCI compared to patients with thoracic SCI in the supine and upright position. Although, in our case, HF (nu) was significantly greater in the cervical SCI group compared to controls during HUTT only.

Regarding the LF/HF, previous finding reported conflicting results. Inoue et al. showed that in quadriplegics the LF/HF power ratio was significantly larger as compared with control group. Claydon and Krassioukov [43] instead, demonstrated that the LF/ HF ratio was lower in the cervical SCI group than in control and thoracic SCI groups in both supine and upright positions. In the upright position, LF/HF ratio increased in the control group but did not increase significantly in the cervical SCI or thoracic SCI group. Finally, in study by Grimm et al. [7], LF/HF ratio was found normal. Our study demonstrated that cervical SCI during supine rest had a normal LF/HF ratio compared to controls, suggesting that, despite the interruption of the sympathetic outflow, these patients are able to maintain homeostasis in supine position. Though, during the orthostatic challenge, due to the inability to increase HR via sympathetic chronotropic efferences or increased cardiac baroreflex delay, tetraplegics' LF/HF was significantly reduced compared to controls.

Limitations

This study was designed to evaluate cardiovascular control in individuals with SCI under controlled laboratory conditions. However, we must acknowledge that, even if patients were asked to delayed their morning dose until after the experiment and not to assume any medication from the midnight preceding the study, the effect of drugs on cardiovascular parameters could not be completely ruled out. Furthermore, one patient (Pt 12) was on intrathecal baclofen and morphine and could not suspend his treatment. All our subjects were male, therefore we cannot provide any evidence on female SCI patients. Patients and controls were asked to lay down relaxed on the tilt table and not to sleep or talk during the study. However, we did not measure EMG activity, so it is possible that some controls activated the leg muscle pump during the orthostatic challenge and patients may have presented involuntary contractions due to spasticity. However all the recording were visually inspected and artifacts were excluded from analysis.

Conclusions:

Non-invasive cardiovascular and sudomotor testing allows to detect the presence and quantify the extension of autonomic dysfunction in SCI patients. Our results confirm previous findings demonstrating that abnormal cardiovascular regulation is correlated with the level of SCI. Cardiovascular autonomic dysfunction, including orthostatic hypotension, was common in tetraplegics, while paraplegics presented near to normal cardiovascular reflexes. The assessment of HRV demonstrated that tetraplegics and paraplegics despite the interruption of the sympathetic outflow, are able to maintain homeostasis in supine position. During HUTT instead, cervical SCI demonstrated an unbalanced sympatho-vagal modulation during HUTT while paraplegics presented near to normal responses.

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Section II.2: The circadian rhythm of blood pressure and heart rate in SCI patients

Background:

The autonomic nervous system (ANS) is believed to play an integral role in the maintenance of 24-hour cardiovascular hemodynamics, and changes in sympathetic nerve activity (SNA) relate to daytime versus night-time heart rate (HR), and blood pressure (BP) [1]. It has been reported that the post-ganglionic sympathetic nerve activity and blood pressure in normal subjects are both lower during deep non-rapid eye-movement (NREM) sleep than they are while subjects are awake [1]. Arousal stimuli during NREM sleep elicit K complexes, accompanied by bursts of sympathetic nerve activity and a transient increase in blood pressure. While the subject is awake, BP and SNA are elevated under psychologic stress and during exercise but are low during rest [2]. Consequently, the circadian profile of SNA resembles that of the BP [3]. The interruption of the pathway that links the brain and the spinal sympathetic neurons can therefore alter the circadian variation of BP.

Reductions in nocturnal SNA, HR, and BP and relative increases in vagal tone [4] during NREM sleep are thought to be cardioprotective. Absence or diminution of nocturnal reductions in HR, BP, and SNA are associated with increased incidence of stroke [5], congestive heart failure [6], and cardiovascular morbidity [7]. Therefore, 24-h, daytime and nighttime variation in cardiovascular hemodynamics and autonomic function may provide insight into long-term cardiovascular health and/or disease risk.

Cardiovascular disease (CVD) has emerged as a leading cause of mortality in persons with chronic spinal cord injury (SCI), exceeding renal and pulmonary complications [8-10]. Increased morbidity due to CVD, especially coronary heart disease (CHD), is reported in individuals with SCI [9] and the incidence of CVD may occur earlier in life compared to the non-SCI population [10].

Few previous studies assessed the 24-h circadian rhythm of BP and heart rate (HR) in SCI patients [11-17]. Furthermore, all these studies were performed by means of ambulatory blood pressure and/or heart rate recordings and therefore, assessed the day-night differences in cardiovascular parameters but not the proper circadian rhythm. Finally these studies were limited to small groups of patients [13,17], mainly tetraplegics [16-18] and/or were performed in uncontrolled settings [11,12,16,17].

In a recent ambulatory study on 20 tetraplegics, 10 paraplegics T2–T5, 9 paraplegics T7–T12, and 10 controls [11] revealed that daytime systolic blood pressure (SBP) was lower in the tetraplegics group compared to the control and paraplegic groups. Although the mean nighttime SBP was not significant in the four groups, the nocturnal dip in SBP was significantly diminished in the tetraplegics group compared to the other groups. No differences were observed between T2–T5 and T7–T12 paraplegics and controls. Similar results were demonstrated in previous studies by Munakata et al. [15], and Nitsche et al.[16]. In this study [11] daytime and night-time HR were similar in tetraplegics and controls groups, but the nocturnal dip in HR was significantly reduced in tetraplegics compared to controls. Similar HR changes were described by Munakata et al. [15], but inconsistent findings regarding the presence/absence of a HR circadian rhythm in tetraplegics were previously reported [16,17].

Objective:

The evidence of increased CVD risk in persons with paraplegia and the paucity of literature focused on 24-h cardiovascular hemodynamic and autonomic function in these individuals have prompted this study. The objectives were: 1) to compare the results of a 24-h continuous recording of BP and HR values in SCI

individuals and able-bodied controls under controlled environmental conditions; 2) to examine the impact of the lesion level on the circadian modulation of BP and HR by comparing subjects with a cervical and thoracic lesion. Furthermore, we aimed to assess the interaction between the sleep cycle and cardiovascular parameters in patients with SCI, comparing BP and HR values between sleep phases (NREM sleep, REM sleep) and wake (state-dependent modulation).

Materials and Methods:

Subjects

Patients and controls were the same of Section I (pag 6) and Table I.1 (pag 7).

The investigation conformed with the principles outlined in the Declaration of Helsinki [19]. The protocol was approved by the Institutional Review Board of the University of Bologna. All participants provided informed consent.

Study protocol

Systolic and diastolic blood pressure (DBP), heart rate, and wake-sleep cycle were continuously monitored for 24-h from 11:00 am SBP, DBP and HR were monitored beat-to-beat with a Portapres portable recorder (Portapres® Model-2, Finapres Medical Systems, Paasheuvelweg, Amsterdam, Netherland). The sleep-wake cycle was monitored by an ambulatory polygraphic recorder (Albert Grass Heritage®, Colleague TM PSG Model PSG16P-1, Astro-Med, Inc, West Warwick, RI, USA or Neurofax Electroencephalograph EEG-1200, Nihon-Kohden Corp., Tokyo, Japan) recording electroencephalogram (EEG: C3-A2, C4-A1, O2-A1), right and left electro-oculogram, electrocardiogram, electromyogram of the mylohyoideus and left and right anterior tibialis muscles and thoraco-abdominal breathing. During the study subjects were allowed to sleep ad libitum, living in a temperature (24°±1°C) and humidity (40-50%) controlled room, lying in bed except when eating, in a light-dark schedule (dark period: 11:00 pm-7:00 am). The subjects were placed on a 1.800 kcal/day diet divided into three meals (8:00 am, 12:00 am, 6:00 pm) and three snacks (10:00 am, 4:00 pm, 11:00 pm). From midnight preceding the monitoring, subjects were instructed to avoid alcohol and caffeinated beverages and to abstain from smoking.

Sleep parameters

The 24-h sleep-wake cycle starting from 11:00 am on the first day of PSG recording was visually scored in 30 s epochs according to the standardized criteria of Rechtschaffen and Kales [20] as light (stages N1 and N2) non-REM sleep, deep (stage N3) NREM sleep, and REM sleep.

Time-dependent analysis

For each subjects, we analyzed the distribution over the 24-h period of the mean value of SBP, DBP and HR calculated every 15 min.

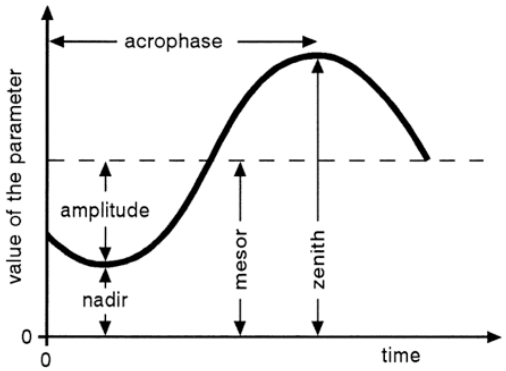
Day-night pattern of SBP, DBP and HR

To evaluate the nocturnal decline of SBP, DBP and HR, daytime mean values (from 7:00 am-11:00 pm) and night-time mean values (from 11:00 pm-7:00 am) were calculated [21,22]. The difference between night-time and daytime values (ΔSBP, ΔDBP, ΔHR) was also calculated.

24-h circadian rhythm of SBP, DBP and HR

Rhythmicity was analyzed by evaluating the time series for SBP, DBP and HR according to the single cosinor method (Fig II.2.1), using a computerized procedure [23]. The procedure determined whether or not there was a rhythm with a 24-h period (p<0,05) and evaluated the following parameters of the cosinor function with their 95% confidence limits: the mesor (Midline Estimating Statistic of Rhythm: 24-h mean), the amplitude (AMP: one-half the peak to trough distance of the approximated waveform) and the acrophase (ACR: peak time referred to local midnight hour). For each patient (except patient 12) we analyzed the 24-h rhythmicity of a single day, starting at 11:00 am. For all controls subjects and patient 12, we analyzed the 24-h rhythmicity of two consecutive days, the first (day 1) starting at 11:00 am, and the second (day 2) starting at 8:00 am.

Fig II.2.1: rhythmicity analyzed according to the single cosinor method



State-dependent changes in SBP, DBP and HR

The analysis was conducted on the data of the 24-h period of PSG recording. The mean value of each variable in each sleep stage (NREM stage 1, 2, 3 and REM sleep) was calculated. The difference (Δ) between the mean value of each variable in any sleep stage and the mean value in wake (W), considered as the reference value, was calculated.

Statistical analysis

Data are reported as means ± SD. Between subjects data, which were normally distributed, were compared using a repeated measures analysis of variance (ANOVA) with Bonferroni and Dunnett's T3 post hoc tests for multiple comparisons as appropriate. Between subjects analysis from not normally distributed data, were compared using the Kruskal–Wallis test. State-dependent analysis of cardiovascular parameters was performed by mixed factorial ANOVA where the factors were wake-sleep phases (W, NREM stage 1 and 2, SWS, REM) and group (cervical SCI *vs* thoracic SCI and controls subjects). All statistical analyses were performed with IBM SPSS Statistics version 20.0 and a p<0,05 was considered significant.

Results:

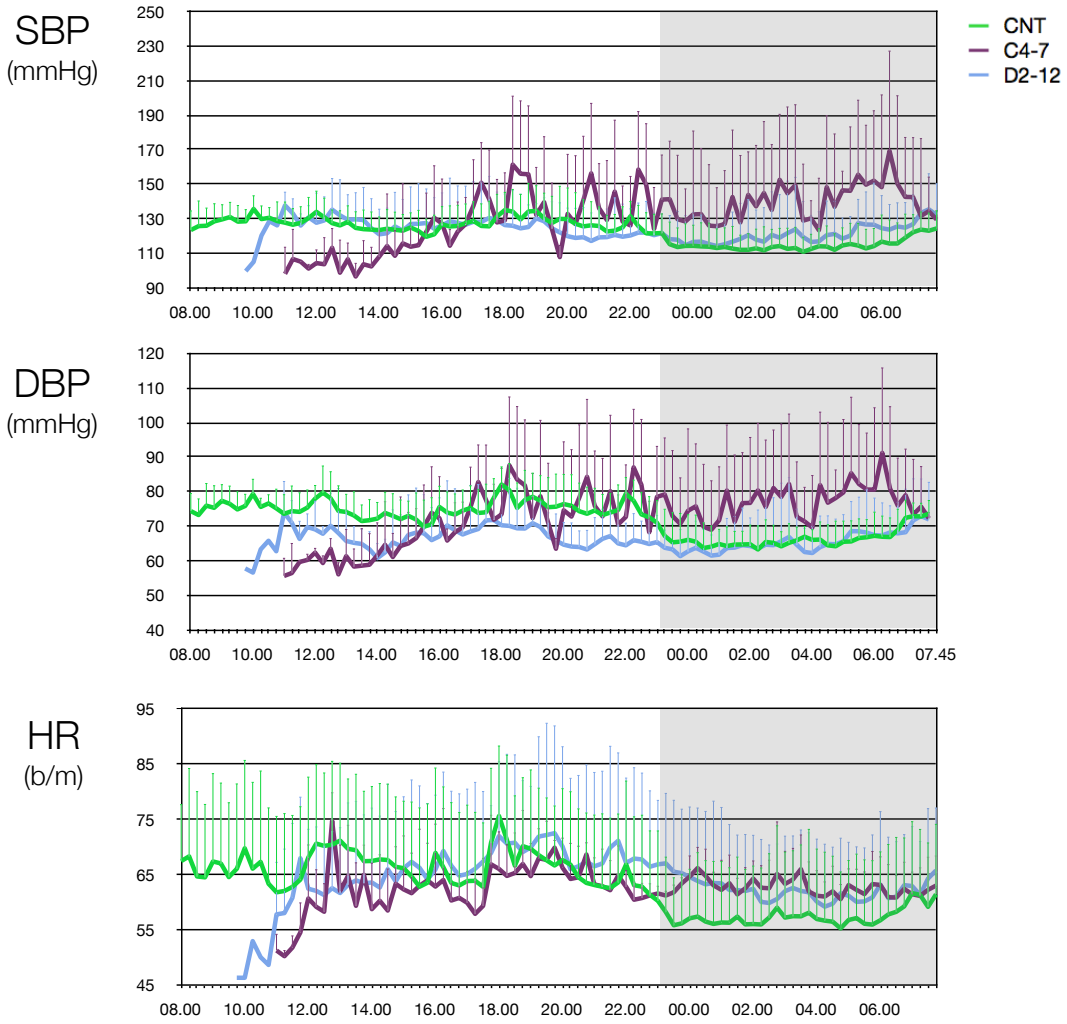
Subjects characteristics

See Section 1 (pag 7).

Time-dependent analysis

Cervical SCI compared to controls presented significantly lower SBP values during the late morning-early afternoon (11:00 am-1:00 pm), but significantly higher SBP values during the night (1:00 am to 6:30 am) (Fig II. 2.2). Similarly, cervical SCI compared to controls had significantly lower DBP values around noon (12:00 pm-2:00 pm), but significantly higher DBP values during the second part of the night (3:00 am to 6:00 am) (Fig II.2.2). Thoracic SCI SBP pattern was similar to controls, with a blunted physiological BP decline during night-time. Thoracic SCI DBP instead, compared to controls, was significantly lower during the late morning-early afternoon (11:00 am-3:00 pm), but similar to controls (with no evident physiological BP decline) during the night. HR pattern was highly variable during the daytime and not statistically different in the three groups. Although, during the night, cervical SCI did not display the physiological decline and HR values were significantly higher in cervical SCI compared to controls from 11:00 pm to 3:00 am (Fig II.2.2). Thoracic SCI HR pattern was half-way from the two other groups (Fig II.2.2).

Fig II.2.2: 24-h pattern of SBP, DBP and HR in SCI patients and controls



Day-night pattern of SBP, DBP and HR

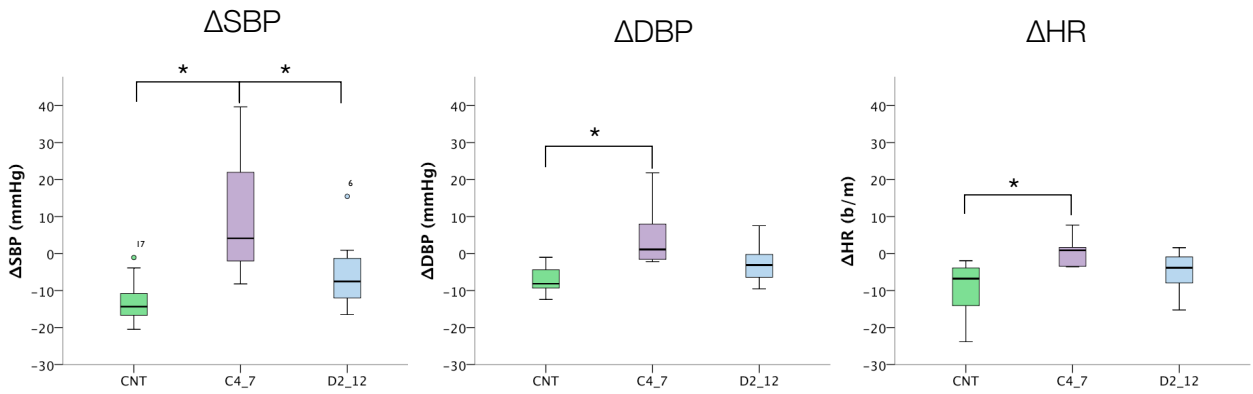
The day-night pattern of SBP, DBP and HR is summarized in Table II.2.1. Daytime and night-time SBP, DBP and HR mean values were comparable in SCI and CNT subjects (p>0,05). Although, tetraplegic patients demonstrated a significant night-time increase in SBP (ΔSBP), DBP (ΔDBP) and HR (ΔHR), while controls presented the physiological decline in cardiovascular parameters (ΔSBP p=0,001; ΔDBP p=0,001; ΔHR p= 0,019) (Fig II.2.3 and Fig II.2.4). Thoracic SCI presented a modest reduction in SBP during the night which was significantly different from cervical SCI ΔSBP (p=0,033). No statistically significant differences in ΔDBP and ΔHR were observed between paraplegics and tetraplegics.

Table II.2.1 : mean daytime, night-time and day-night changes (Δ) in SBP, DBP and HR in SCI and controls

Condition	Parameter	CNT (n=14)	C4-7 (n=5)	D2-12 (n=8)
Daytime	**SBP (mmHg)**	127±10	127±18	125±15
	DBP (mmHg)	75±5	71±10	67±8
	HR (b/m)	66±13	62±6	67±13
Night-time	**SBP (mmHg)**	114±11	138±35	120±16
	DBP (mmHg)	67±5	77±18	65±9
	HR (b/m)	57±11	63±3	62±11
Δ (night-time-daytime)	**SBP (mmHg)**	-13±5*	11±20	-5±10*
	DBP (mmHg)	-7±3*	5±10	-3±5
	HR (b/m)	-9±7*	1±5	-5±5

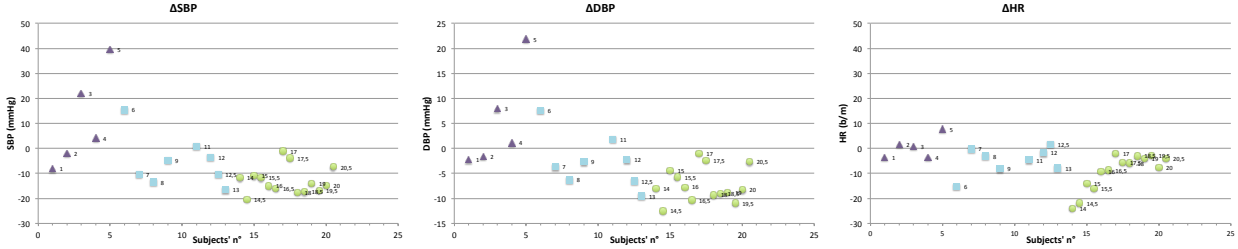
Values are means ± SD. *=p<0,05 vs. cervical SCI.

Fig II.2.3: mean day-night changes (Δ) in SBP, DBP and HR in SCI patients and controls



⋆=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

Fig II.2.4: day-night changes (Δ) in SBP, DBP and HR in SCI patients and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

Three (Pt 3, 4 and 5) out of five cervical SCI displayed a “reverse dipper” SBP pattern, while the remaining two (Pt 1 and 2) had a “non dipper” pattern. Among the seven thoracic SCI, two (Pt 6 and 11) displayed a “reverse dipper” SBP pattern, three (Pt 7, 9 and 12) were “non dipper” and two (Pt 8 and 13) were “dipper”. A “non dipper” SBP pattern was detected only in one (17) control subject on both nights.

24-h circadian rhythm of SBP, DBP and HR

SBP mesor (CNT: 127±10 mmHg; C4-7: 131±24 mmHg; D2-12: 124±15; p= 0,789) DBP mesor (CNT: 72±4 mmHg; C4-7: 73±13 mmHg; D2-12: 67±9; p= 0,193) and HR mesor (CNT: 63±12 mmHg; C4-7: 62±4 mmHg; D2-12: 65±12 mmHg, p= 0,722) were comparable in the three groups.

The AMP of SBP rhythm (CNT: 9±2 mmHg; C4-7: 16±12 mmHg; D2-12: 10±5 p= 0,561), DBP (CNT: 5±2 mmHg; C4-7: 7±7 mmHg; D2-12: 5±3 p=0,918) and HR (CNT: 6±4 mmHg; C4-7: 3±2 mmHg; D2-12: 4±4 p=0,113) was not significantly different in SCI patients and controls.

The ACR values of SBP (CNT: 14:52±136’; C4-7: 9:28±564’; D2-12: 14:12±308’; p=0,389), DBP (CNT: 14:44±188’; C4-7: 10:04±564’; D2-12: 11:00±320’; p=0,209) and HR (CNT: 15:00±108’; C4-7: 11:56±572’; D2-12: 15:00±348’; p=0,722) were comparable in the three groups.

Table II.2.2 : results of circadian rhythm analysis of SBP, DBP and HR in SCI patients and controls

Circadian rhythm of		CNT (n=14)	C4-7 (n=5)	D2-12 (n=8)
SBP	**Mesor (mmHg)**	123±10	131±24	124±15
	Amplitude (mmHg)	9±2	16±12	10±5
	Acrophase (h:mm±min)	14:52±136’	9:28±564’	14:12±308’
DBP	**Mesor (mmHg)**	72±4	73±13	67±9
	Amplitude (mmHg)	5±2	7±7	5±3
	Acrophase (h:mm±min)	14:44±188’	10:04±564’	11:00±320’
HR	**Mesor (b/m)**	63±12	62±4	65±12
	Amplitude (b/m)	6±4	3±2	4±4
	Acrophase (h:mm±min)	15:00±108’	11:56±572’	15:00±348’

Values are means ± SD.

State-dependent changes in SBP, DBP and HR during night-time

Night-time SBP, DBP and HR mean values during the REM and NREM sleep stages (Tab II.2.3; Fig II.2.5) were comparable in SCI and CNT subjects (p>0,05), with the exception that SBP during REM sleep was significantly higher in cervical SCI compared to thoracic SCI and controls (CNT: 116±12 mmHg; C4-7: 162±32 mmHg; D2-12: 121±19; C4-7 vs CNT: p= 0,001; C4-7 vs D2-12: p=0,004).

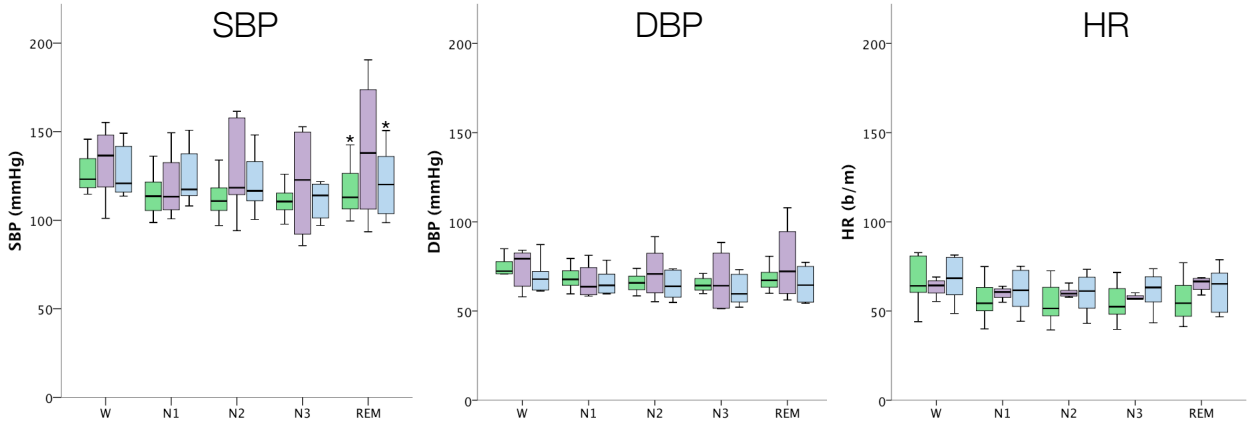
However, while within subjects statistical analysis revealed a physiological state-dependent modulation of BP and HR in able-bodies participants (Tab II.2.3; Figure II.2.6), characterized by a BP and HR reduction during NREM sleep with respect to wake (W) values during the light period (SBP, DBP and HR: p<0,001 in all comparisons) and by their increase during REM sleep with respect to NREM sleep (SBP: p<0,02, DBP: p=0,03, HR: p=0,01) [24], a similar trend in SBP, DBP and HR values was not detected in both groups of SCI patients. Tetraplegics and paraplegics did not display any significant change in BP values between wake, REM and NREM sleep (p>0,05), except for thoracic SCI who showed a significant reduction in HR values during stage 1 (N1) and 2 (N2) NREM sleep compared to wake (W) (p<0,01).

Table II.2.3 : state dependent changes in SBP, DBP and HR in SCI patients and controls

Parameter		CNT (n=14)	C4-7 (n=5)	D2-12 (n=8)
SBP (mmHg)	**W**	127±10	132±22	127±14
	N1	116±12°	119±21	125±16
	N2	112±10°	129±29	121±16
	N3	111±8°	121±34	111±10
	REM	116±12*,°	162±32	121±19*
DBP (mmHg)	**W**	75±4	74±12	69±9
	N1	68±6°	67±10	66±7
	N2	66±5°	72±15	65±8
	N3	65±4°	67±19	62±8
	REM	68±6°	77±23	65±10
HR (b/m)	**W**	66±13	63±6	68±12
	N1	56±11°	60±4	62±11°
	N2	55±11°	61±3	60±11°
	N3	54±10°	58±2	61±11
	REM	56±11°	65±4	62±12

Values are means ± SD. *=p<0,05 vs. cervical SCI. °=p<0,05 vs within group wake (W) values

Fig II.2.5: state dependent changes in SBP, DBP and HR in SCI and controls (between groups)



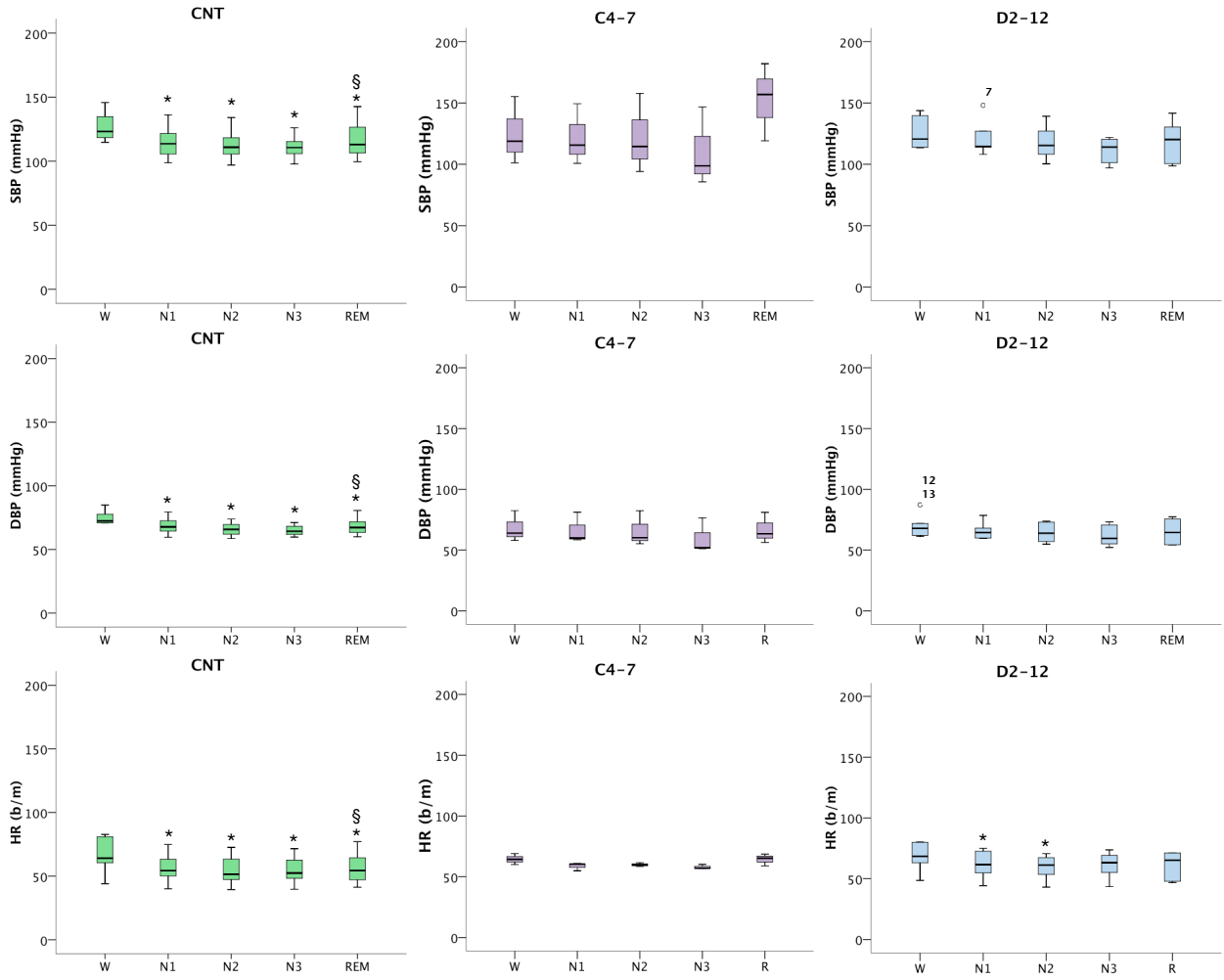
*=p<0,01 vs. cervical SCI.

C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

Discussion:

To our knowledge this is the first study which assessed the 24-h circadian rhythm in individuals with tetraplegia and paraplegia with continuos recording of cardiovascular parameters under controlled environmental conditions. Furthermore, this is the first study which evaluated the state-dependent modulation of BP and HR in SCI patients and healthy controls.

Fig II.2.6: state dependent changes in SBP, DBP and HR in SCI and controls (between groups)



✶=*p*<0,05 *vs* within group wake (W) values; §=p<0,03 *vs* within group stage 3 (N3) NREM sleep values

C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

Time dependent analysis of cardiovascular parameters demonstrated that cervical SCI compared to controls display a reversed circadian pattern with lower BP values during the day and higher BP values during the night (inverse dipper). Cervical SCI also tended to present higher HR values during the night compared to controls, while daytime values were similar in the two groups. Thoracic SCI SBP trend was similar to controls' but presented a blunted nocturnal dip. Paraplegics daytime DBP tended to be lower than controls, had a blunted nocturnal dip and therefore night-DBP values were similar between thoracic SCI and controls. No significative differences were observed between thoracic SCI HR trend and controls.

Daytime and night-time SBP, DBP and HR mean values were comparable in SCI and CNT subjects, but tetraplegic patients demonstrated a significant night-time increase in SBP, DBP and HR, while controls presented the physiological reduction in cardiovascular parameters. Compared to previous findings [11,15], we did not observe significantly lower daytime mean BP values in cervical SCI compared to able-body subjects. Although time-dependent analysis proved that daytime BP values, were significantly lower in tetraplegics compared to able-body subjects in the late morning-early afternoon. This difference may be due to various factors, like the well known BP variability in tetraplegics patients [12-14] or differences in the study protocol (in our patients and controls were lying in bed except when eating, and were allowed to sleep ad libitum); or

concomitant medications. Therefore, despite this discrepancy, we confirmed the previous findings demonstrating reversed day-night BP changes in cervical SCI and a blunted nocturnal BP dip in paraplegics.

Regarding HR day-night variations, previous studies provided conflicting results. Nitsche et al. reported that complete and incomplete tetraplegic patients preserved the HR day-night physiological modifications [16], while according to Munakata et al. and Rosado Rivera et al. the day-night differences in HR were significantly reduced in cervical SCI compared to thoracic SCI and controls. In a previous study on 8 tetraplegic subjects Christ reported that only 5 patients demonstrated the HR day-night physiological modifications, while 3 didn't [17]. In our study daytime and night-time mean HR were similar in the three groups, but the nocturnal decline in HR was reduced in tetraplegics compared to controls. Furthermore, time dependent analysis revealed that cervical SCI tended to present higher HR values between 11:00 pm and 3:00 am compared to controls.

The state dependent analysis of SBP, DBP and HR changes during the night-time confirmed that tetraplegics did not present the physiological state-dependent modulation of BP and HR, as observed in controls, characterized by BP and HR reduction during NREM sleep with respect to wake values and by their increase during REM sleep with respect to NREM sleep [24]. Furthermore this analysis revealed that even paraplegics did not present the state-dependent modulation of BP and HR during sleep, despite their near to normal daytime and night-time cardiovascular changes.

Previous studies attributed the reported BP changes to the autonomic dysfunction resulting from the SCI, which determined an absent increase in sympathetic activity during daytime hours in tetraplegics, and to the preservation of physiological nocturnal dipping of BP values in controls [15,16]. This hypothesis was strengthened by the finding of Casiglia et al., who demonstrated that in able-bodied subjects leg resistance was significantly higher during waking hours (when the sympathetic system is more activated) than during sleep, while in subjects with spinal cord injury no difference was detected between day-time and night-time [14]. Similarly, the differences in 24-h HR between tetraplegics and controls were attributed to the disruption of sympathetic chronotropic efferents to the heart in high SCI lesions and to the physiological lowering of HR values during sleep in controls [25]. Although, as demonstrated in Section II.1, the autonomic control of the cardiovascular system is severely affected in cervical SCI but near to normal in thoracic SCI. Therefore, the observed alteration in 24-h BP an HR profile, at least in thoracic SCI, cannot be explained solely by the changes in SNA due to the SCI. Other pathogenetic mechanism may be involved. It is tempting to speculate that the reduction in slow wave sleep we observed in both cervical and thoracic SCI (see Section I) could play a role in altering the 24-h BP and HR profile in these patients, similarly to what was previously described in obstructive sleep apnea (OSA) [26,27] or other disorders causing sleep fragmentation [28]. Whether these finding are the direct consequence of SCI or secondary to the sleep related breathing disorders frequently associated with this condition is still to be determined.

Previous studies demonstrated an increased risk of CVD and mortality in SCI patients [10,29,30]. The increased risk for CVD is not limited to tetraplegic patients, but there is evidence suggesting that individuals with paraplegia are at increased risk for CVD [31], silent ischemia [32], and CHD [33], and the relative risk of CHD can also be 70% greater in individuals with chronic paraplegia compared to individuals with chronic tetraplegia [9]. Furthermore, a recent series of publications found that a third of the Swedish paraplegic population is at greater risk for CVD disease than age-matched non-SCI controls [34-36]. Therefore is tempting to speculate that a reverse/absent or blunted BP nocturnal dip and a failure of the state-dependent modulation of cardiovascular parameters may predispose individuals with tetraplegia and paraplegia to an increased risk of CVD and mortality.

Finally, between subjects state dependent analysis showed that tetraplegics, paraplegics and controls presented comparable SBP, DBP and HR mean values during the REM and NREM sleep stages, with the

exception that SBP during REM sleep was significantly higher in cervical SCI compared to thoracic SCI and controls. REM sleep in humans is characterized by a great variability of BP and HR together with breathing irregularities, depending directly on central changes in the regulation of the autonomic outflow that activated peripherally controlled variables. BP rises are sudden, irregular and often wide. During REM, muscle sympathetic nerve activity increases above the levels recorded during wakefulness and this increase is more pronounced than the changes in HR and BP [1,37]. Three out of five cervical patients had a confirmed diagnosis of OSA and one patient reported sleep related breathing disorders. Therefore, we could speculate that the increase in SBP observed during REM sleep in cervical SCI could be related to the breathing irregularities which are prominent during this sleep stage and to the associated mechanical factors and muscle spasms.

Limitations:

Although the study was performed under controlled environmental conditions a possible limitation of the study is that episodes of autonomic hyperreflexia related to spasm, bladder distention or other stimuli may have occurred during the recording, especially in cervical SCI. Furthermore for ethical reason we could not discontinue patients medications and the possible effect of drugs should be taken in account. Finally, because the study population was comprised of men with complete spinal cord injuries, applicability of the findings to females or a cohort of individuals with incomplete SCI cannot be ascertained.

Conclusions:

Overall, our results confirm previous ambulatory findings which demonstrate that day-night BP changes are abolished/reversed in cervical SCI and blunted in thoracic SCI. Consistent with some previous findings we did not observe any HR day-night variations in the three groups but tetraplegic patients presented higher HR values during the night compared to controls. Furthermore, thanks to state dependent analysis, we demonstrated that both cervical and thoracic SCI did not display the physiological modulation of BP and HR between wake, NREM and REM sleep. Whether these findings are the direct consequence of the autonomic dysfunction due to the SCI or secondary to the sleep alterations observed in these patients (see Section I) is still to be determined. Although, due to the possible relation with increased CVD and mortality cardiovascular autonomic scrutiny should be an integral part of SCI examination and the assessment of the 24-h circadian rhythm of SBP, DBP and HR should be brought in clinical practice. Finally, studies should be designed to investigate the short-term and long-term effects of various interventions (e.g. exercise training, pharmacological interventions) on normalization of 24-h cardiovascular and autonomic function in individuals with SCI.

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ALMA MATER STUDIORUM
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Section III

BODY CORE TEMPERATURE REGULATION IN SPINAL CORD INJURY PATIENTS

"Something's wrong. I'm hot. No, wait, now I'm cold. But why am I sweating? I never sweat. My legs won't stop jumping. Now my hands are in tight fists and my torso is tight as well. Why does my scalp itch? Oh no, the headache is starting. I'm getting dysreflexic."

Kim Anderson

Section III: BODY CORE TEMPERATURE REGULATION IN SPINAL CORD INJURY PATIENTS

Background:

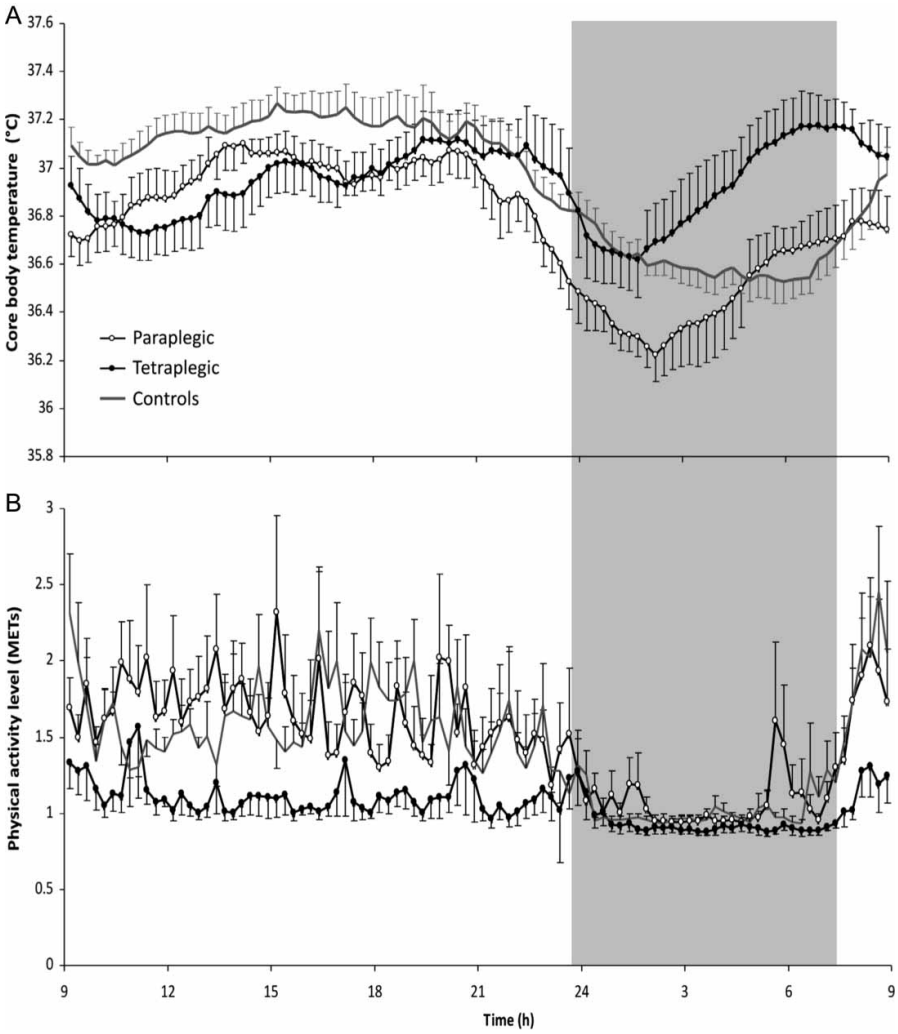
The circadian rhythm of body core temperature (BcT) in diurnally active humans shows a nadir in the morning between 4:00 and 6:00 am and a peak 1–4 h before habitual bedtime [1]. The main temperature regulator in the hypothalamus depends on afferent feedback to adequately regulate BcT [2,3], and relies on the peripheral nervous system to produce heat via muscle activity [4] or to elicit heat loss through vasodilatation and sweat gland activity [5]. If the afferent and/or efferent arms of the sympathetic nerve system are lost, regulation of BcT may be impaired [6,7].

Dependent on the site and extent of the lesion, individuals with a spinal cord injury (SCI) have an abnormal cardiovascular regulation and sudomotor dysfunction below the level of the lesion [8] resulting in an impaired afferent input to, and efferent signals from the central thermoregulatory center [9,10]. Consequently SCI individuals depend on the region above the lesion for thermoregulation [11,12] and may present a thermal dysfunction [6,11,13]. Indeed, hypothermia may occur in patients with high SCI as shivering is diminished and they may be unable to vasoconstrict the cutaneous circulation compared with subjects with a lower lesion [6,11,13]. The reverse may occur causing hyperthermia because of the inability to sweat and to reflexly vasodilate in the periphery, so as to lose heat [14].

Interestingly, thermoregulatory and hypnic mechanisms are thought to be linked [15]. Humans usually initiate sleep on the downward slope of the circadian body temperature rhythm [16], when the rate of change of core temperature and peripheral heat loss are maximal and when endogenous levels of melatonin begin to rise and cortisol decline [17,18]. Body heat loss before lights off, via selective vasodilatation of distal skin regions, promotes the ability to initiate and maintain sleep [19]. Therefore it could be hypothesized that the impaired thermoregulatory function in SCI patient, causing an altered circadian variation of BcT may affect the sleep-wake cycle. Indeed, previous studies have identified a high prevalence of sleep disorders in SCI [20]. The elevated 15–40% prevalence of sleep disorders in SCI individuals [21-24] is higher than the ~4% prevalence typically reported in middle-aged men [25]. Furthermore, nocturnal release of melatonin, a hormone that contributes to the normal sleep pattern [26], contributes to the 24-h variation of BcT. The neural pathway for the endogenous production of melatonin passes through the cervical spinal cord [26,27] and, therefore, may be altered or even absent in SCI individuals with a cervical lesion [28,29].

However, so far only a single recent study examined, with formal chronobiological analyses, the hypothesis that an altered circadian variation of BcT is present in SCI (Fig III.1) [30]. The authors measured continuously and simultaneously intestinal BcT (telemetry system) and physical activity (ambulatory activity monitor) levels in 8 tetraplegics, 7 paraplegics, and 8 able-bodied controls during a 24-h period of “normal” living. In tetraplegics, the mean dominant period length was 6–8 h shorter (p=0,02), whereas the mean BcT during the night-time was significantly higher (p=0,005). Tetraplegics showed a 5-h phase-advanced circadian trough time (p=0,04), and more variable relationships between physical activity and BcT (p=0,03). Taken together these data indicate that tetraplegics demonstrate a pronounced disturbance of the circadian variation of BcT, whereas the variation of BcT in paraplegics was comparable to able-bodied controls.

Fig III.1: 24-h rhythm of BcT and physical activity levels in SCI patients and able-bodied subjects



From Thijssen et al. (2011) Chronobiology international 28 (2):146-154.
24-h rhythm of BcT (A) and physical activity (B) levels in able-bodied subjects (–), paraplegic (○), and tetraplegic (•) subjects. The grey region represents the average time of sleep for all subjects. BcT and physical activity recording started at 9:00 h and was performed continuously for a 24-h period. Data are presented as the mean over a 15 min period, whereas error bars represent SE. Physical activity level was measured using an activity monitor, which calculated the metabolic equivalent (MET), representing the fold change in baseline metabolic rate. Note that the mean MET level during the awake hours is 1.5, whereas during sleep MET levels are 1. Moreover, no significant differences between groups for physical activity level were found.

Objective:

Aims of the study were to compare the BcT during a 24-h recording of SCI individuals and able-bodied controls, and to examine the impact of lesion level on the circadian modulation of BcT by comparing subjects with a high (cervical) and low (thoracic) lesion. Furthermore we aimed to assess the interaction between the sleep cycle and BcT in patients with SCI, comparing BcT values between sleep phases (NREM sleep, REM sleep) and wake (state-dependent modulation).

Materials and Methods:

Subjects

Patients and controls were the same described in Section I (pag 6) and Table I.1 (pag 7).

The investigation conformed with the principles outlined in the Declaration of Helsinki [31]. The protocol was approved by the Institutional Review Board of the University of Bologna. All participants provided written informed consent.

Study protocol

BcT and wake-sleep cycle were continuously monitored for 24-h from 11:00 am. Rectal temperature was monitored every 2 minutes by a Mini-loggerTM portable device. The sleep-wake cycle was monitored by an ambulatory polygraphic recorder (Albert Grass Heritage®, Colleague TM PSG Model PSG16P-1, Astro-Med, Inc, West Warwick, RI, USA or Neurofax Electroencephalograph EEG-1200, Nihon-Kohden Corp., Tokyo, Japan) recording electroencephalogram (EEG: C3-A2, C4-A1, O2-A1), right and left electrooculogram, electrocardiogram, and electromyogram of the mylohyoideus, left and right anterior tibialis muscles and thoraco-abdominal breathing. During the study subjects were allowed to sleep ad libitum, living in a temperature (24 ± 1 °C) and humidity (40-50%) controlled room, lying in bed except when eating, in a light-dark schedule (dark period: 11:00 pm-7:00 am). The subjects were placed on a 1.800 kcal/day diet, divided into three meals (8 am, 12:00 am, 6:00 pm) and three snacks (10:00 am, 4:00 pm, 11:00 pm). From midnight preceding the monitoring, subjects were instructed to avoid alcohol and caffeinated beverages and to abstain from smoking.

Sleep parameters

The 24-h sleep-wake cycle starting from 11:00 am on the first day of polysomnographic (PSG) recording was visually scored in 30 s epochs according to the standardized criteria of Rechtschaffen and Kales [32] as light (stages N1 and N2) non-REM sleep (NREM), deep (stages N3) NREM sleep, and REM sleep.

Time-dependent analysis

We compared the mean value of BcT, calculated every 15 min in each participant, in search for between groups significative differences.

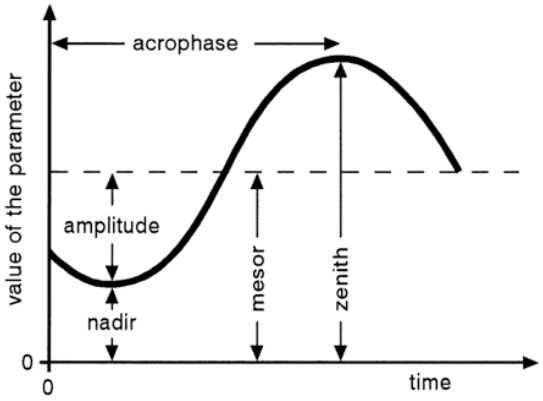
Day-night pattern of BcT

To evaluate the day-night pattern of BcT daytime mean values (from 7:00 am to 11:00 pm) and night-time mean values (from 11:00 pm to 7:00 am) were calculated [33,34]. The nocturnal decline of BcT was determined by the difference between night-time and daytime values (ΔBcT).

24-h circadian rhythm of BcT

Rhythmicity was analyzed by evaluating the time series for BcT according to the single cosinor method, using a computerized procedure (Fig III.2) [35]. The procedure determined whether or not there was a rhythm with a 24-h period (p<0,05) and evaluated the following parameters of the cosinor function with their 95% confidence limits: the midline estimating statistic of rhythm (mesor: 24-h mean), the amplitude (AMP: one-half the peak to trough distance of the approximated waveform) and the acrophase (ACR: peak time referred to local midnight hour). For each patient (except patient 12) we analyzed the 24-h rhythmicity of a single day, starting at 11:00 am. For all controls subjects and patient 12, we analyzed the 24-h rhythmicity of two consecutive days, the first (day 1) starting at 11:00 am, and the second (day 2) starting at 8:00 am.

Fig III.2: rhythmicity analyzed according to the single cosinor method



State-dependent changes in BcT

The analysis was conducted on the data of the 24-h period of PSG recording. The mean value of each variable in each sleep stage (NREM stage 1, 2, 3 and REM sleep) was calculated. The difference (Δ) between the mean value of BcT in any sleep stage during the 24-h period and the mean value in wake (W), considered as the reference value, was also calculated.

Statistical analysis

Data are reported as means ± SD. Between subjects data, which were normally distributed, were compared using a repeated measures analysis of variance (ANOVA) with Bonferroni and Dunnett's T3 post hoc tests for multiple comparisons as appropriate. Between subjects analysis from not normally distributed data, were compared using the Kruskal–Wallis test. State-dependent analysis of BcT was performed by mixed factorial ANOVA where the factors were wake-sleep phases (W, NREM stage 1, 2, 3 and REM) and group (cervical SCI *vs* thoracic SCI and controls subjects). All statistical analyses were performed with IBM SPSS Statistics version 20.0 and a $p<0,05$ was considered significant.

Results:

Subjects characteristics

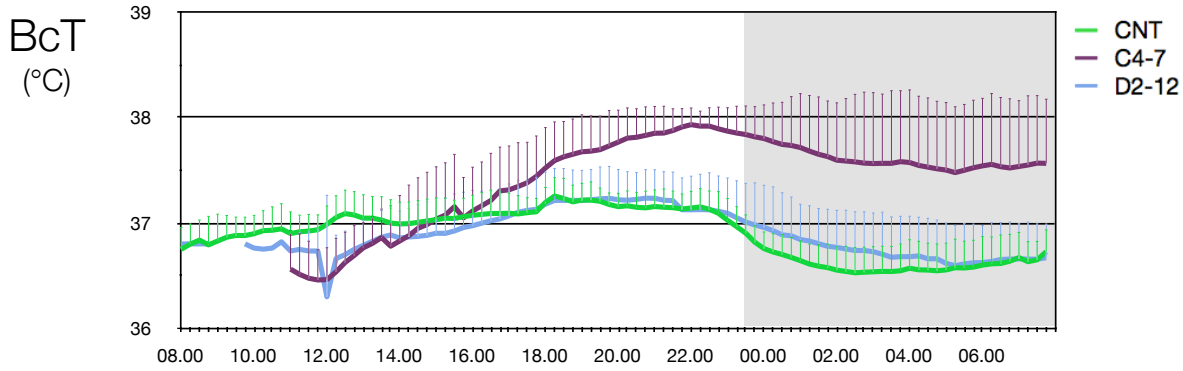
See Section 1 (pag 7).

All patient underwent sympathetic skin response (SSR) testing before enrollment to assess neurogenic activation of sweat glands. SSR was proposed as useful tool to investigate autonomic involvement in SCI, and, in addition to motor and sensory evaluation, may improve classification of the extent of spinal functional deficits [36]. As expected all cervical SCI had absent SSR in all limbs. Thoracic SCI patients 7 to 12 had absent SSR in lower limbs and normal SSR in upper limbs. Patient 6 had absent SSR in all limbs, while patient 13 had normal SSR in all limbs and sacral region

Time-dependent analysis

Cervical SCI presented a reversed pattern of 24-h BcT (non-dipper) (Fig III.3) with, compared to controls, significantly lower values around noon and significantly higher BcT values from 5:00 pm to 7:00 am. Thoracic SCI presented a 24-h BcT trend similar to controls.

Fig III.3: 24-h pattern of BcT in SCI patients and controls



BcT: body core temperature; C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

Day-night pattern of BcT

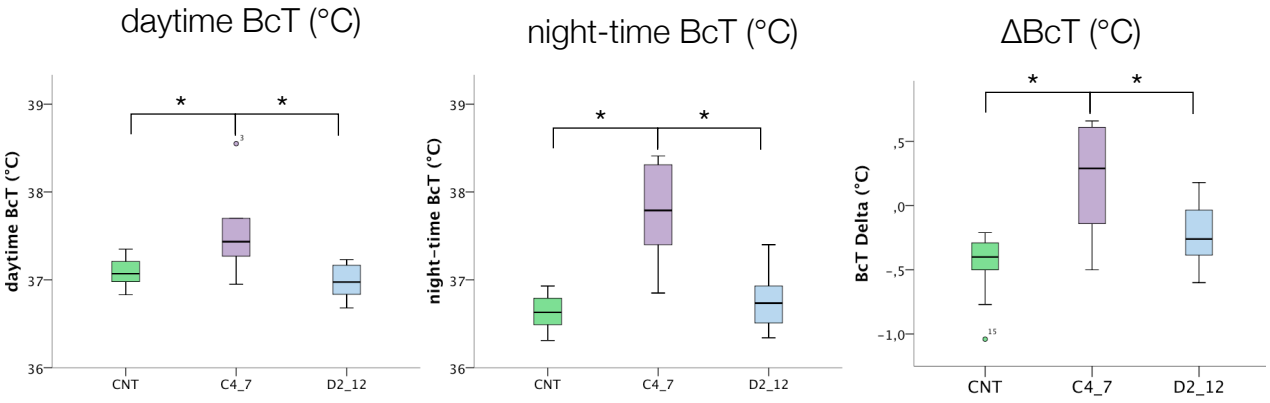
The 24-h pattern of BcT is shown in Fig III.3 and summarized in Table III.1, Fig III.4 and Fig III.5. Daytime and night-time BcT mean values were significantly higher in cervical SCI compared to thoracic SCI and CNT subjects (p=0,009 and p=0,004 respectively for daytime BcT; p=0,000 and p=0,000 respectively for night-time BcT). The night-time fall in BcT (ΔBcT) (Fig III.4, Fig III.5) was significantly reduced in tetraplegic patients compared to controls and paraplegics (p=0,001 and p=0,048 respectively).

Table III.1: daytime BcT, night-time BcT and day-night changes (Δ) in BcT in SCI and controls

Circadian rhythm of		CNT (n=14)	C4-7 (n=6)	D2-12 (n=8)
BcT (°C)	**Daytime**	37,07±0,16*	37,56±0,55	36,98±0,20*
	Night-time	36,63±0,20*	37,76±0,59	36,76±0,33*
	Δ	-0,44±0,23*	0,2±0,46	-0,22±0,27*

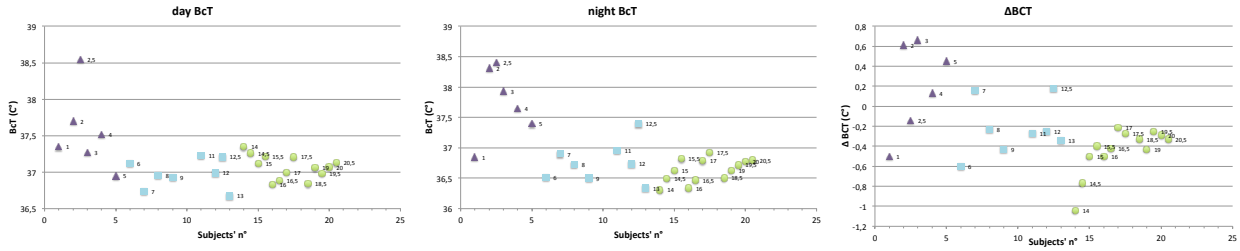
Values are means ± SD. *=p<0,05 *vs* cervical SCI.

Fig III.4: mean daytime, night-time and day-night changes (Δ) in BcT in SCI patients and controls



⋆=p<0,05. BcT: body core temperature; C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; Δ: day-night changes.

Fig III.5: daytime BcT, night-time BcT and day-night changes (Δ) in BcT in SCI patients and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

24-h circadian rhythm of BcT

A reversed circadian rhythmicity of BcT was detected in all SCI patients and controls (Table III.2).
BcT mesor (CNT: 36,92±0,14 °C; C4-7: 37,64±0,49 °C; D2-12: 36,86±0,22 °C) was significantly higher in cervical SCI compared to thoracic SCI and CNT subjects (p=0,011 and p=0,002 respectively).

The AMP of BcT rhythm (CNT: 0,33±0,17 °C; C4-7: 0,45±0,15 °C; D2-12: 0,29±0,14 °C) was comparable in the three groups (p=0,19).

The ACR values of BcT (CNT: 16:52±52'; C4-7: 7:08±492'; D2-12: 16:16±400') was not significantly different in SCI patients and controls (p=0,059)

Table III.2: 24-h circadian rhythm analysis of BcT in SCI patients and controls

Circadian rhythm of		CNT (n=14)	C4-7 (n=6)	D2-12 (n=8)
BcT (°C)	**Mesor (°C)**	36,92±0,14*	37,64±0,49	36,86±0,22*
	Amplitude (°C)	0,33±0,17	0,45±0,15	0,29±0,14
	Acrophase (h:mm±min)	16:52±52'	7:08±492'	16:16±400'

Values are means ± SD. *=p<0,01 *vs* cervical SCI.

State-dependent changes in BcT

The mean values of BcT during the REM and NREM sleep stages (Tab III.3; Fig III.6) were significantly higher in cervical SCI compared to thoracic SCI and CNT subjects (p< 0,01 in all comparison).

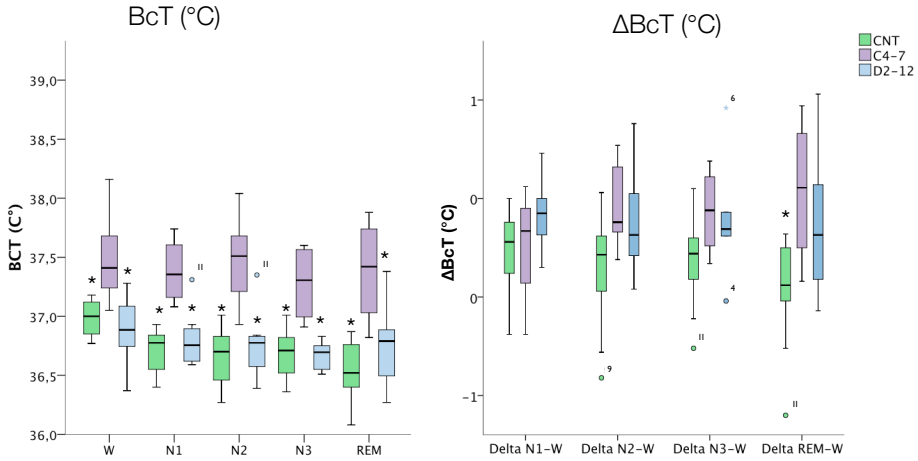
However, while statistical analysis revealed a physiological state-dependent modulation of BcT in control subjects (Tab III.3; Fig III.7), characterized by BcT reduction during REM and NREM sleep with respect to W values (p<0,001) [37], a similar trend was not observed in SCI patients. Both tetraplegic and paraplegics did not present statistically significant changes in BcT during REM and NREM sleep stages compared to W values (p=1 in all comparisons).

Table III.3: state dependent changes in BcT in SCI patients and controls

Parameter		CNT (n=14)	C4-7 (n=5)	D2-12 (n=8)
BcT (°C)	**W**	36,99±0,15*	37,51±0,43	36,89±0,28*
	N1	36,71±0,17*,°	37,38±0,29	36,81±0,24*
	N2	36,64±0,23*,°	37,47±0,43	36,76±0,28*
	N3	36,68±0,19*,°	37,28±0,34	36,67±0,12*
	REM	36,55±0,25*,°	37,39±0,46	36,75±0,34*

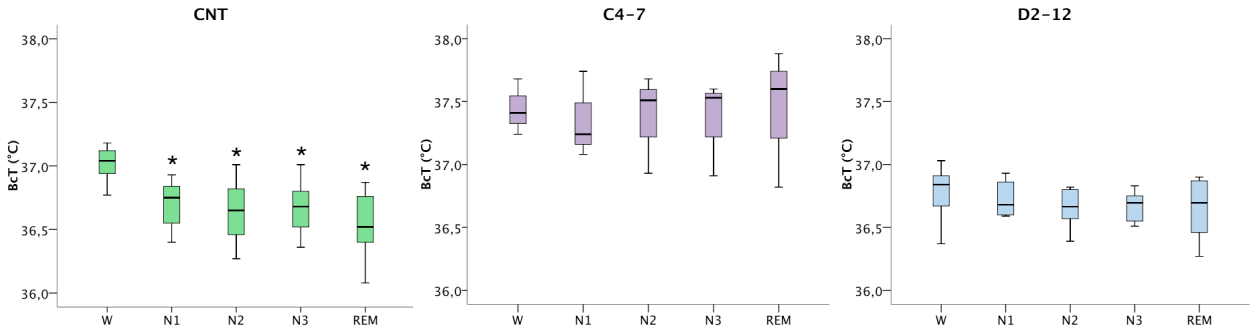
Values are means ± SD. *=p<0,01 *vs* cervical SCI. °=p<0,001 *vs* within group wake (W) values

Fig III.6: state dependent variation of BcT and BcT changes (Δ) with respect to wake in SCI patients and controls (between groups)



*=p<0,01 vs cervical SCI. BcT: body core temperature; C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; Δ: changes with respect to wake; N1: stage 1 NREM sleep; N2: stage 2 NREM sleep; N3: stage 3 NREM sleep; REM: REM sleep; SCI: spinal cord injury; W: wake.

Fig III.7: state dependent changes in BcT in SCI patients and controls (within groups)



*=p<0,001 vs within group wake (W) values
BcT: body core Temperature; C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; N1: stage 1 NREM sleep; N2: stage 2 NREM sleep; N3: stage 3 NREM sleep; REM: REM sleep; SCI: spinal cord injury; W: wake.

Discussion:

This study analyzed the 24-h circadian rhythm and the state-dependent modulation of BcT in SCI patients and healthy controls under controlled environmental conditions.

Cervical SCI compared to controls presented a reversed pattern of 24-h BcT (non-dipper) (Fig III.3) with significantly lower values around noon and significantly higher BcT values from 5:00 pm to 7:00 am. The daytime and night-time BcT mean values were significantly higher in cervical SCI compared to thoracic SCI and CNT subjects, and the night-time fall in BcT was significantly absent/reversed in tetraplegic patients compared to controls and paraplegics. The cosinor analysis of BcT exhibited a significantly higher mesor in cervical SCI compared to thoracic SCI and CNT subjects, while AMP and ACR were comparable in the three groups (even if a tendency for an earlier ACR was observed in cervical SCI compared to thoracic SCI and controls).

In the only one available previous study on BcT variation in SCI, Thijssen et al. [30] reported a higher nocturnal BcT and a 5-h phase-advanced circadian trough time in tetraplegics compared to paraplegics and controls, but did not observe a higher daytime BcT or a higher mesor in cervical SCI as we did. Although, this study assessed

patients during their standard routine activities at their home, and, even if they were required to remain indoors, constant stable environmental condition could not be guaranteed. The current study, instead, was performed under controlled ambient conditions, in order to exclude the well documented influence exerted on BcT rhythm by external factors such as light-dark cycle, food intake, sleep, room temperature, humidity, and body position (the so called photic and non-photic entrainment (zeitgebers) of the human circadian system) [38,39]. The presence/absence of these confounders may explain the discrepancies between their findings and our results. Similarly to Thijssen et al. we did not find any important differences in the paraplegic 24-h variation in BcT compared to controls. Therefore, even if thoracic SCI have a reduced surface area available for thermoregulation, this may be enough to maintain BcT in the physiological range, or different mechanisms, other than skin thermoregulation, may be involved in 24-h regulation of BcT. Based on previous observations demonstrating that the neural pathway for the endogenous production of melatonin passes through the cervical spinal cord [27,29] and that tetraplegics show no nocturnal release of melatonin [28,29] Thijssen et al. hypothesized that these changes in endocrine function may contribute to the observed differences in BcT, especially since tetraplegics demonstrated the most pronounced change in the 24-h variation in BcT. However, no previous study examined contextually melatonin release and BcT in SCI in order to establish a relationship between these two variables. Furthermore, our data demonstrate a 24-h (daytime and night-time) elevation of BcT in cervical SCI compared to thoracic SCI and CNT subjects which may be difficult to relate to the pulsatile secretion of melatonin.

Important new insights were provided by the state-dependent analysis of BcT in our study. This analysis revealed, as expected, that BcT was significantly higher in cervical SCI during wake, NREM and REM sleep stages compared to controls and thoracic SCI. Furthermore, it demonstrated that both tetraplegics and paraplegics did not display the physiological modulation of BcT between wake, NREM and REM sleep during the 24-h. Indeed, BcT was not reduced during REM and NREM sleep with respect to wakefulness in cervical and thoracic SCI patients.

In Section I we discussed the differences occurring in the sleep structure between SCI patients and controls, demonstrating that both cervical and thoracic SCI presented a higher prevalence of light sleep and a reduction in slow wave sleep (SWS) (Section I, Fig I.1, Fig I.2, Table I.3). As demonstrated by previous studies, the sleep disturbances observed in patients with SCI cannot be explained solely by disruptions in breathing during sleep [21,40,41]. Indeed, even if sleep related breathing disorders (SRBD) may exert an important role in determining the higher number of arousals observed in our cervical SCI group (Section I, Fig I.3, Fig I.4, Table I.3) and their increased proportion of light sleep, they could not explain the alteration in sleep architecture observed in thoracic SCI, since paraplegics had a apnea-hypopnea index (AHI) comparable to controls (Section I, Table I.3).

Therefore, other factors may play a role in determining the predominance of light sleep observed in tetraplegics and paraplegics, including their inability to regulate thermal function. Although, the reverse could be also possible, being the reduced SWS to interfere with body temperature changes. Further studies will be necessary to assess the relationship between the sleep disorders and the circadian variation of BcT observed in SCI patients.

Conclusions:

Overall, our results confirm previous findings which demonstrate that tetraplegics present a pronounced disturbance of the circadian variation of BcT. Furthermore, thanks to state dependent analysis, we also demonstrated that both cervical and thoracic SCI did not display the physiological modulation of BcT between

wake, NREM and REM sleep. The pathogenetic mechanism and the possible consequence of such alterations are yet to be determined.

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CONCLUSIONS

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Overall the major findings of the current study are:

1. Chronic cervical and thoracic SCI have a compromised sleep, characterized by an augmented proportion N2 NREM sleep and reduced SWS compared to controls. No statistical differences in any sleep parameter were found between cervical and thoracic SCI patients. While the sleep disturbances in cervical SCI could be related to SRBD, higher AHI, and possibly to an alteration in melatonin secretion, the pathogenesis of the sleep disorder in thoracic SCI is yet to be determined.

2. Abnormal cardiovascular regulation is correlated with the level of SCI. Cardiovascular autonomic dysfunction, including orthostatic hypotension, was common in tetraplegics, while paraplegics presented near to normal cardiovascular reflexes.

3. The assessment of HRV demonstrated that tetraplegics and paraplegics despite the interruption of the sympathetic outflow, are able to maintain homeostasis in supine position. During HUTT instead, cervical SCI demonstrated an unbalanced sympatho-vagal modulation while paraplegics presented near to normal responses.

4. Day-night BP changes are abolished/reversed in cervical SCI and blunted in thoracic SCI. We did not observe any HR day-night variations in the three groups but tetraplegic patients presented higher HR values during the night compared to controls.

5. State dependent analysis of BP and HR changes demonstrated that both cervical and thoracic SCI did not display the physiological modulation of BP and HR between wake, NREM and REM sleep. Whether these finding are the direct consequence of the autonomic dysfunction due to the SCI or secondary to the sleep alterations observed in these patients is still to be determined.

6. Tetraplegics present a pronounced disturbance of the circadian variation of BcT.

7. State dependent analysis of variation of BcT, revealed that both cervical and thoracic SCI did not display the physiological modulation of BcT between wake, NREM and REM sleep. The pathogenetic mechanism and the possible consequence of such alterations are yet to be determined.

Further studies should be designed to investigate the effects of various interventions (e.g. treating OSA, reducing PLMS, ameliorating the thermoregulation, etc...) on the association between sleep alteration, autonomic dysfunction and thermoregulatory failure we documented, in order to detect the presence and the direction of a causal relationship between these parameters.

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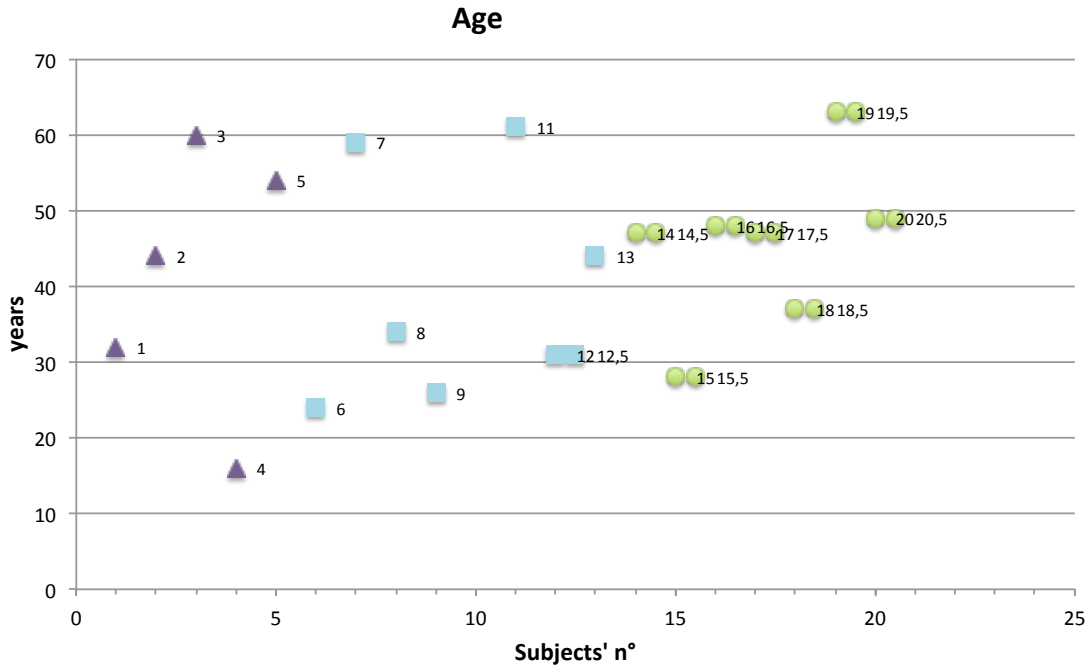
APPENDIX

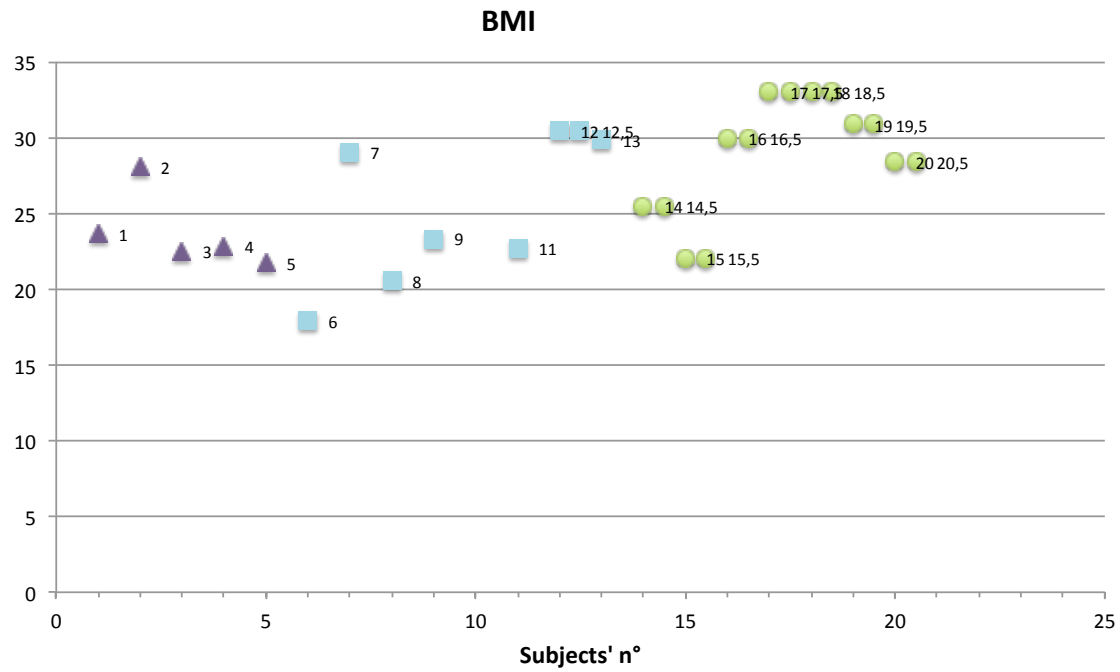
APPENDIX: Section I

Age distribution in SCI patients and controls



▲C4-7 ■D2-12 ●CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

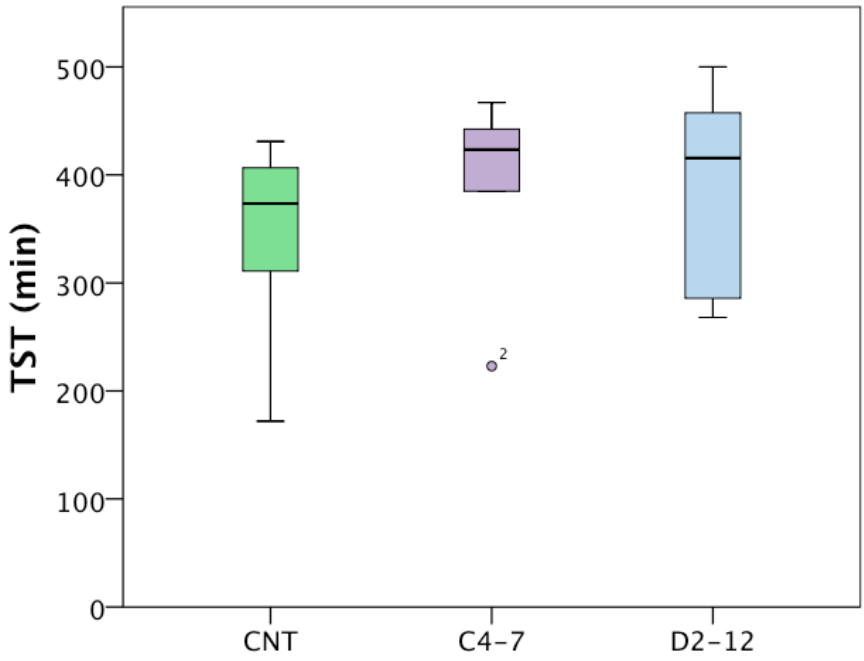
BMI distribution in SCI patients and controls

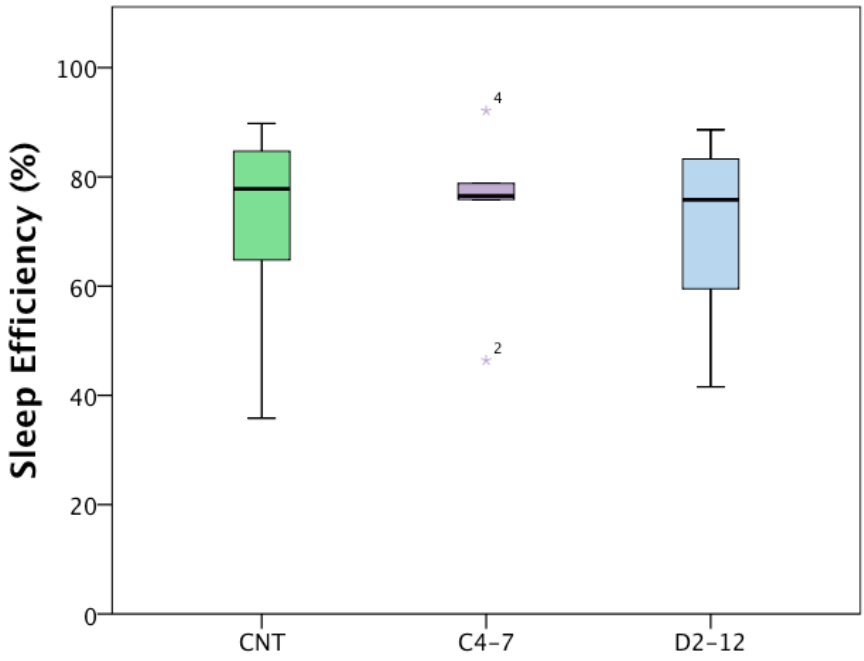


▲C4-7 ■D2-12 ●CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

APPENDIX: Section I

Total Sleep Time (TST) and Sleep Efficiency in SCI patients and controls

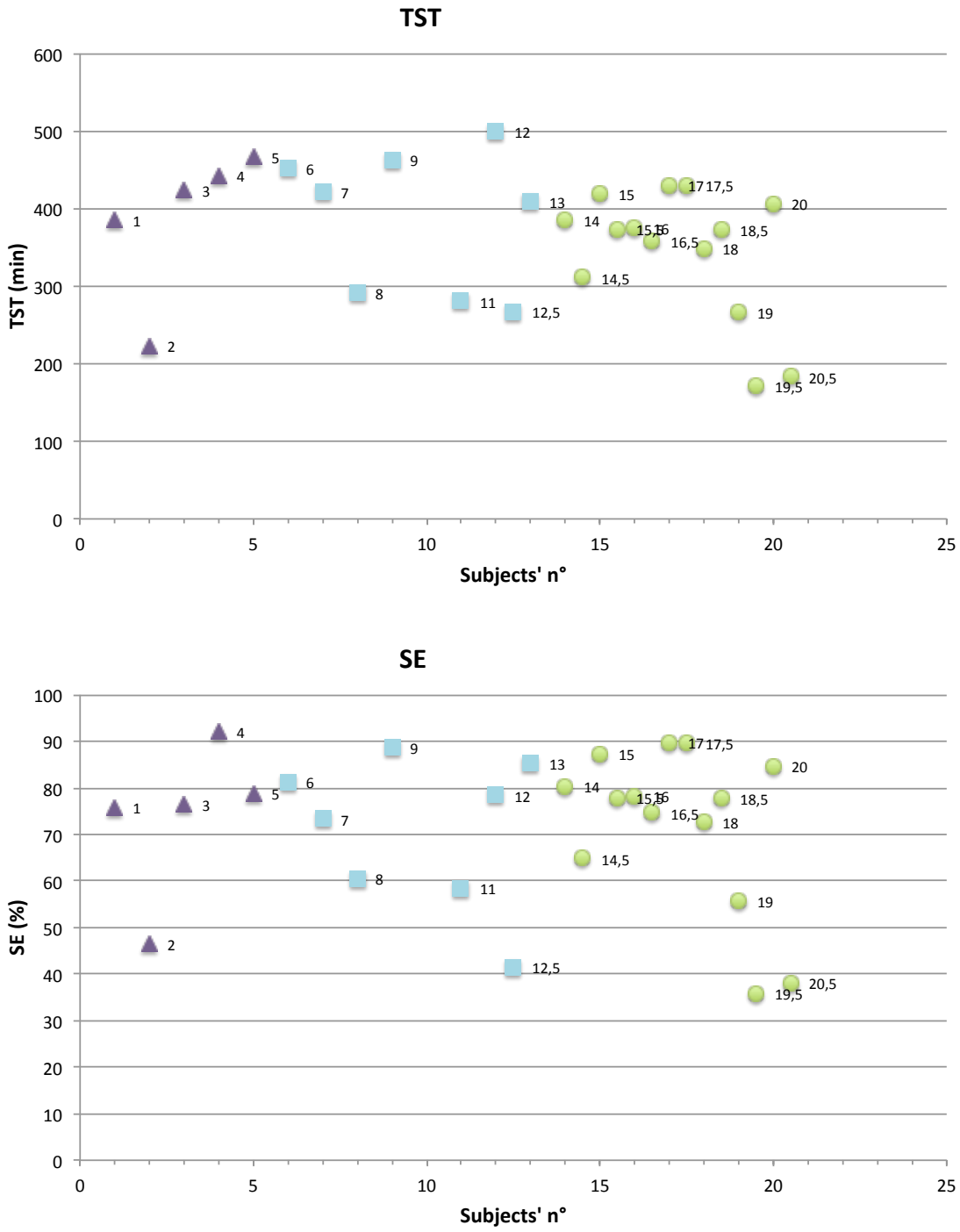




C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

APPENDIX: Section I

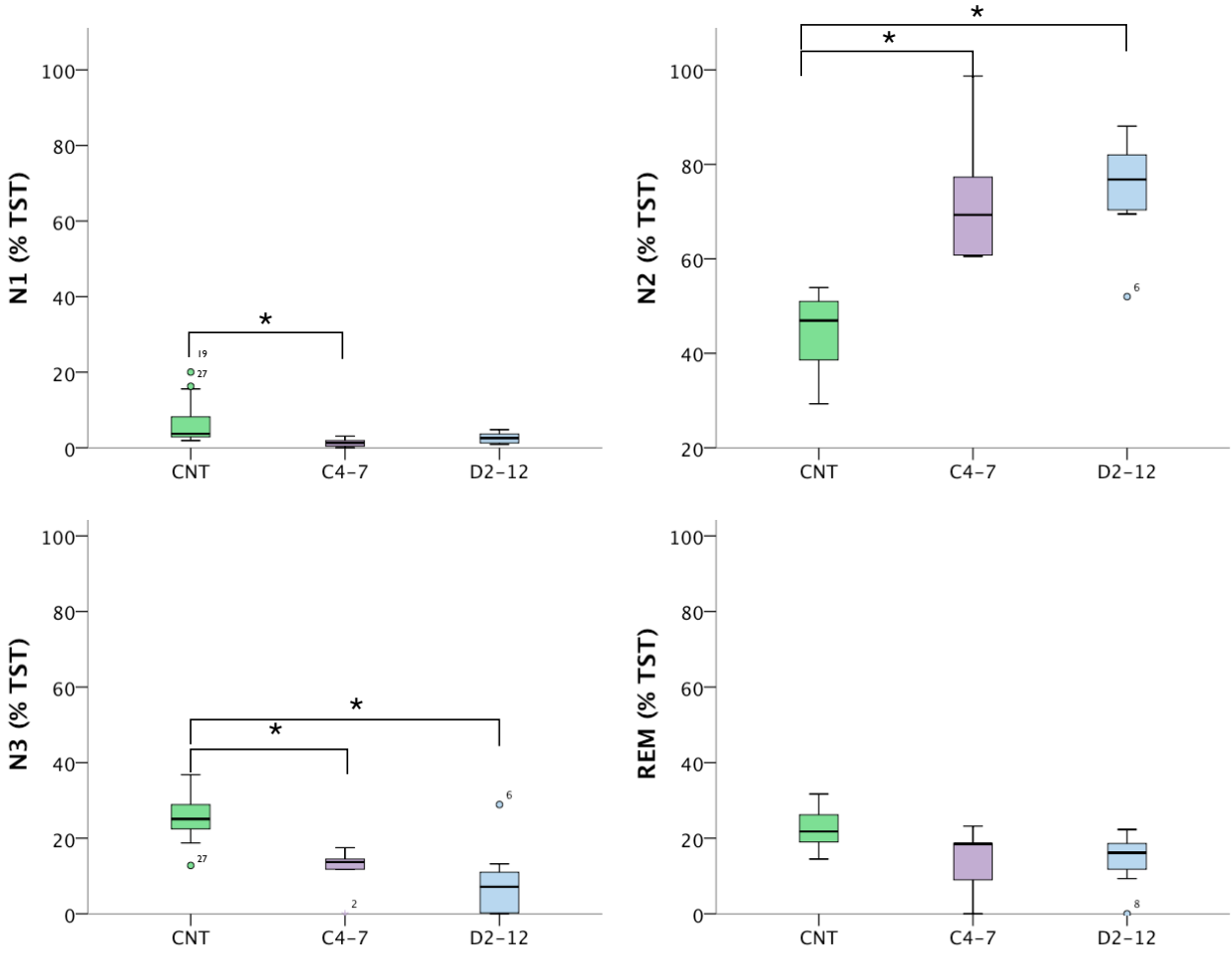
Total Sleep Time (TST) and Sleep Efficiency (SE) in SCI patients and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

APPENDIX: Section I

Fig I.2: Percentage of TST occupied by NREM and REM sleep phases in SCI patients and controls



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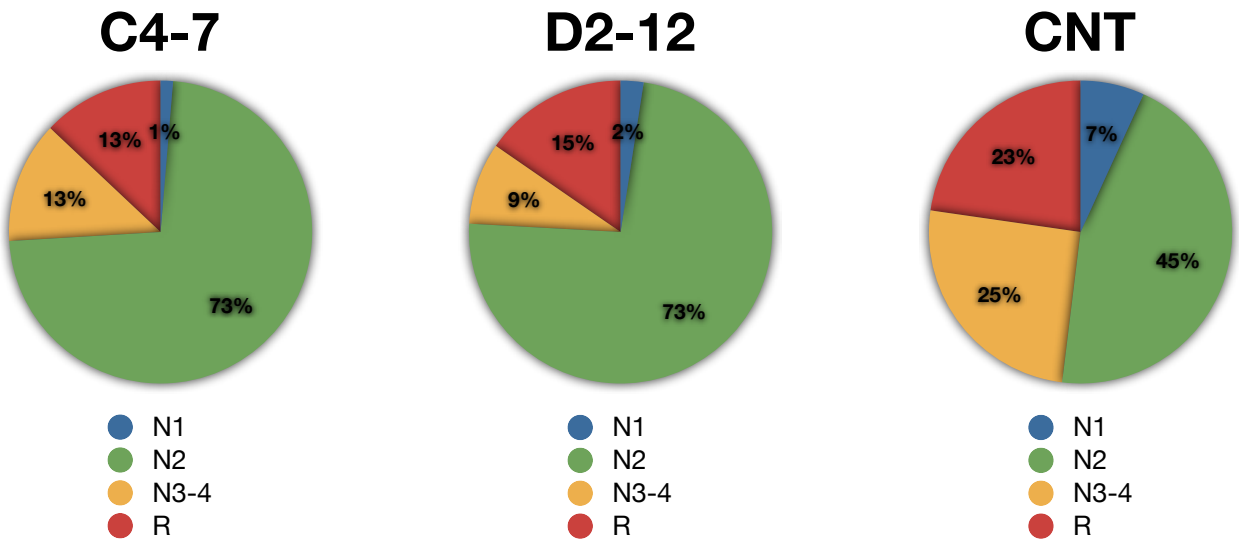
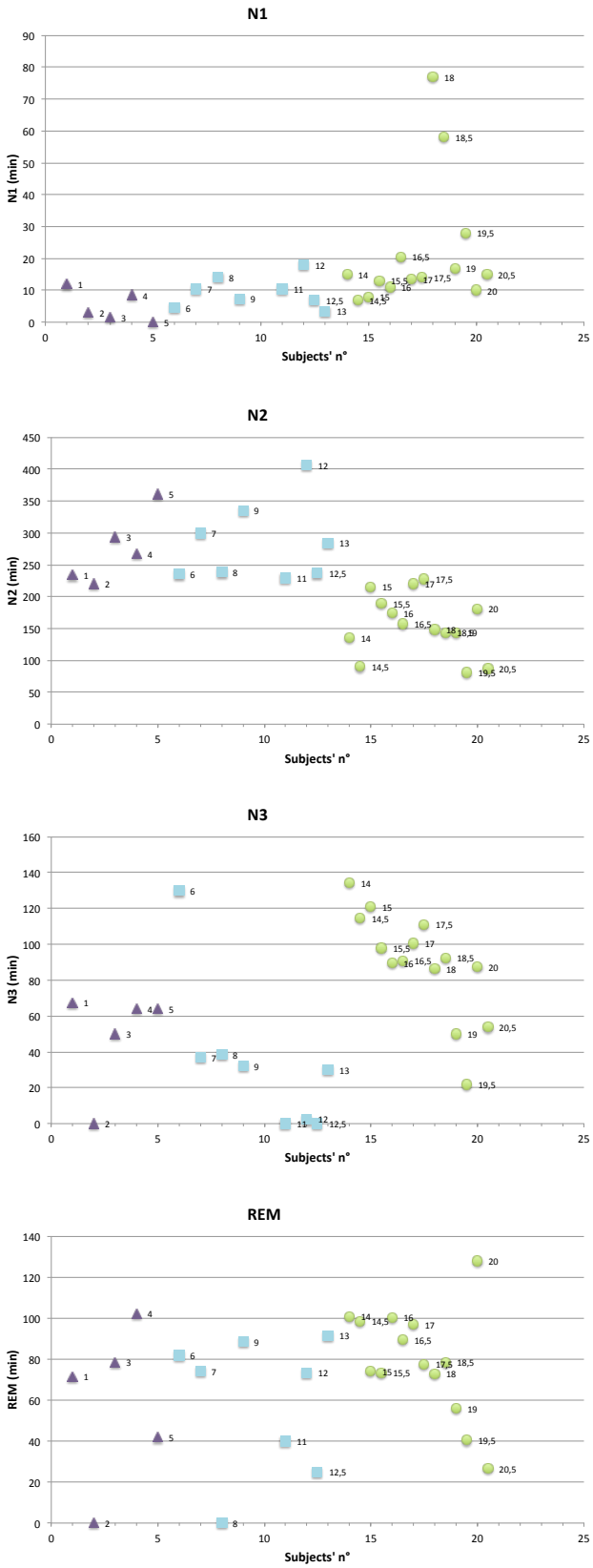


C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; N1: stage 1 NREM sleep; N2: stage 2 NREM sleep; N3: stage 3 NREM sleep; REM: REM sleep; SCI: spinal cord injury; TST: total sleep time.

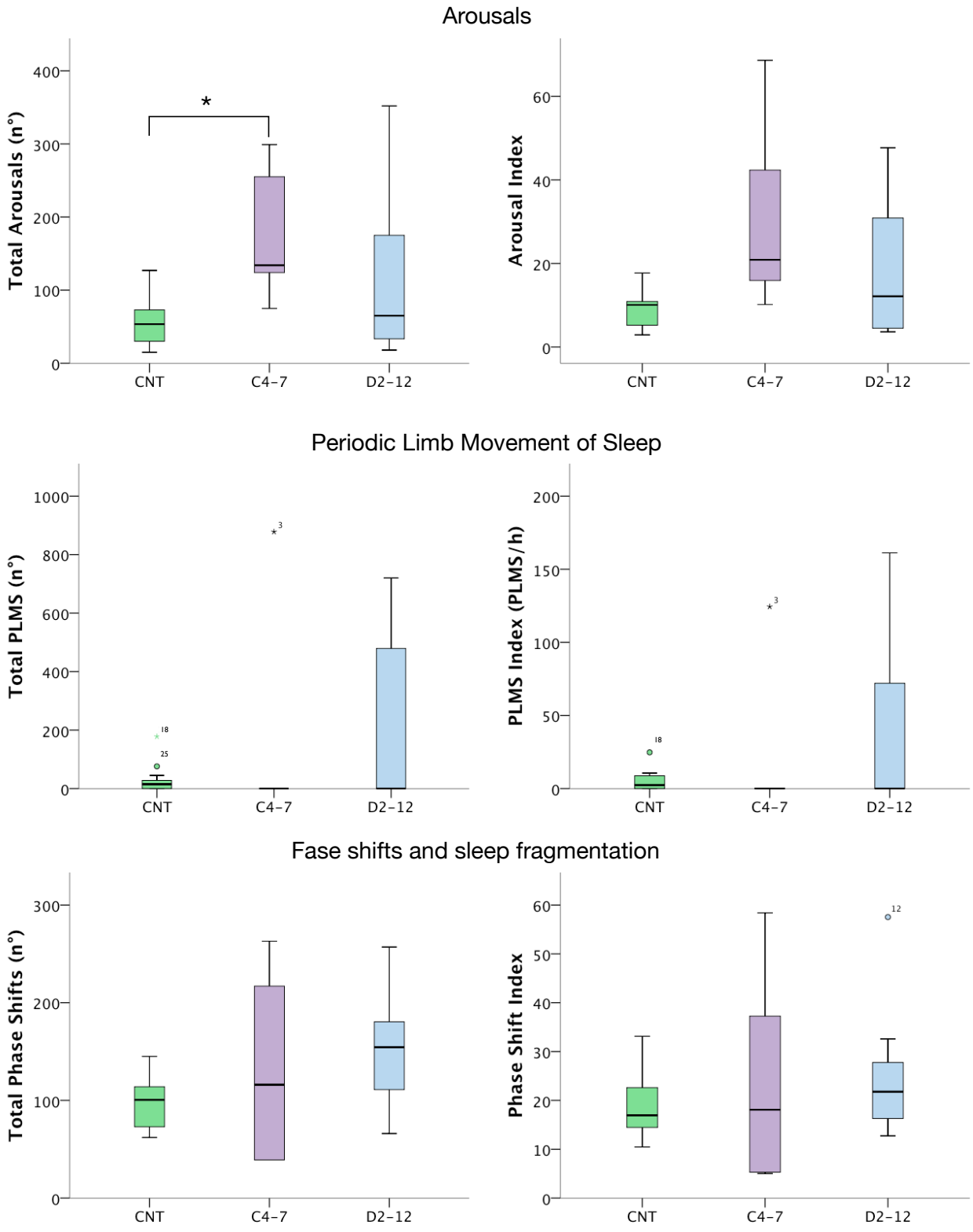
APPENDIX: Section I

Fig I.3: minutes of NREM and REM sleep phases in SCI patients and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; N1: stage 1 NREM sleep; N2: stage 2 NREM sleep; N3: stage 3 NREM sleep; REM: REM sleep; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

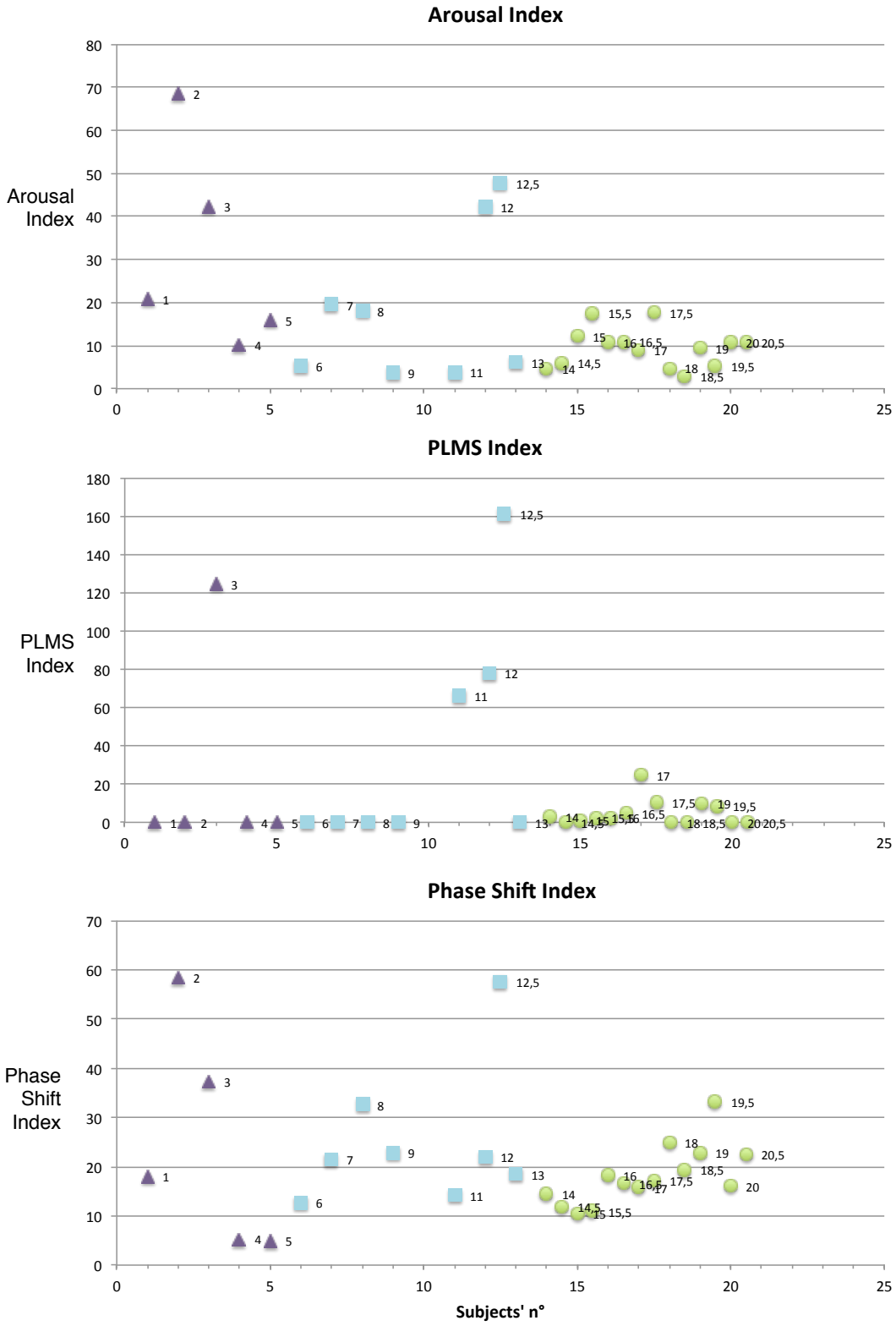
Fig I.4: arousals, periodic limb movement and phase shifts in SCI patients and controls



∗=p<0,05

C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury, PLMS: periodic limb movement during sleep.

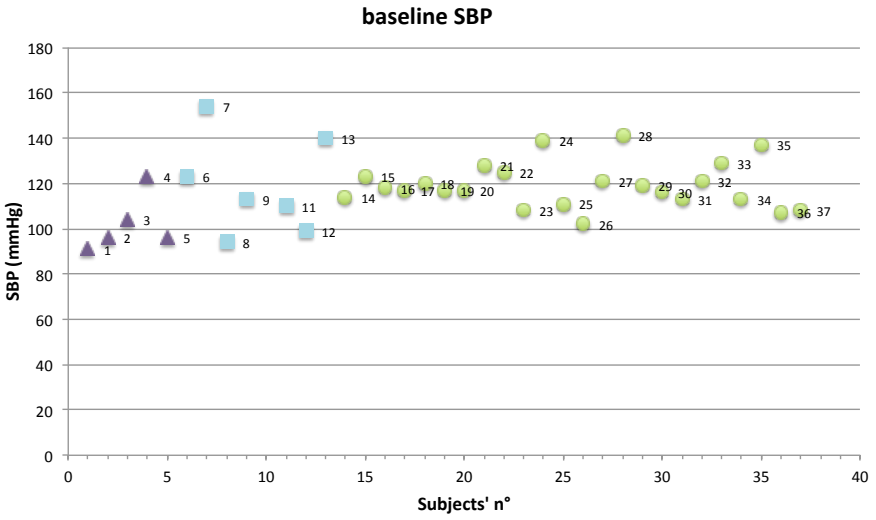
Fig I.5: arousals, periodic limb movement and phase shifts in SCI patients and controls

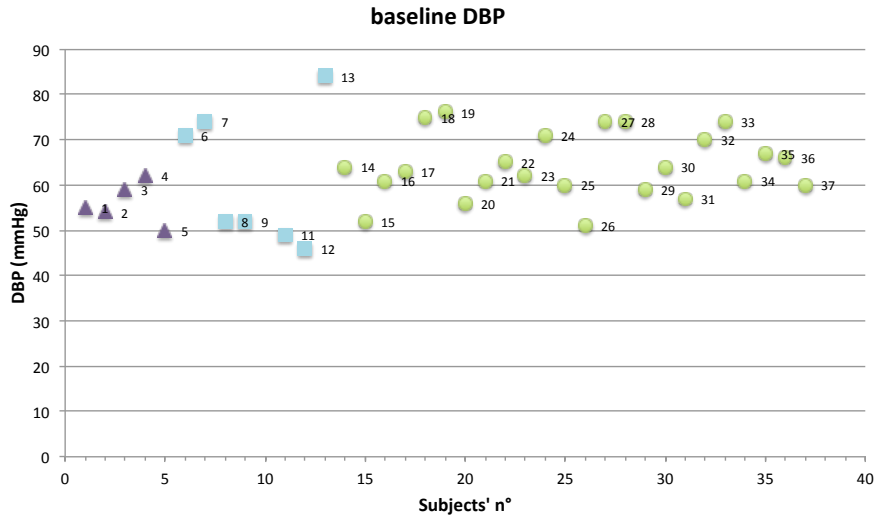


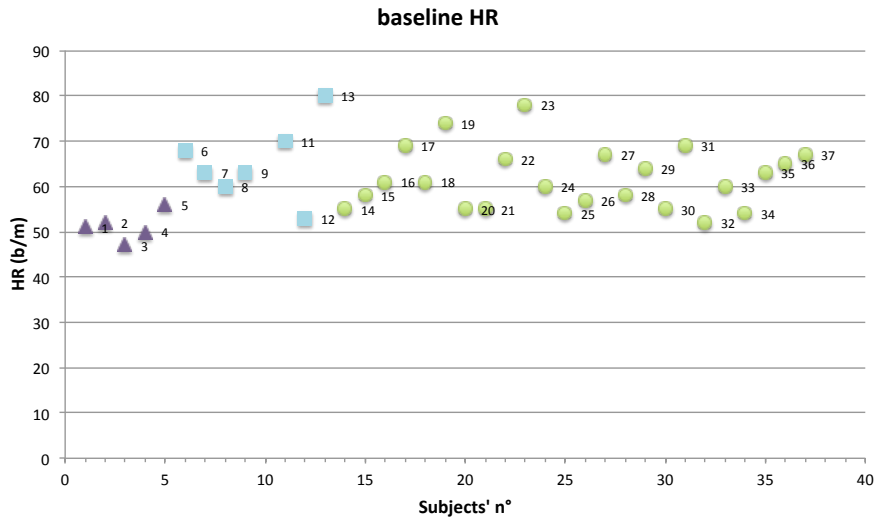
▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; PLMS: periodic limb movement during sleep. Numbers on axis of abscissas correspond to subjects' numbers.

APPENDIX: Section II.1

Supine SBP, DBP and HR values in SCI patients and controls



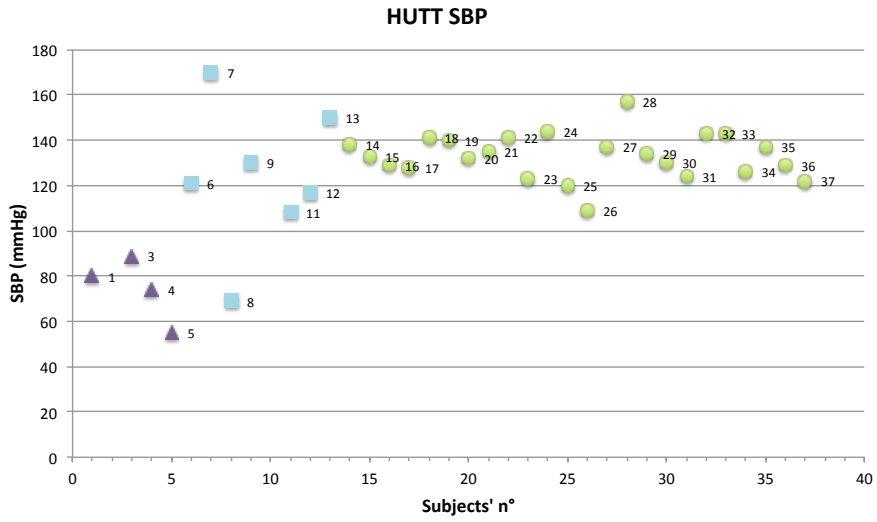


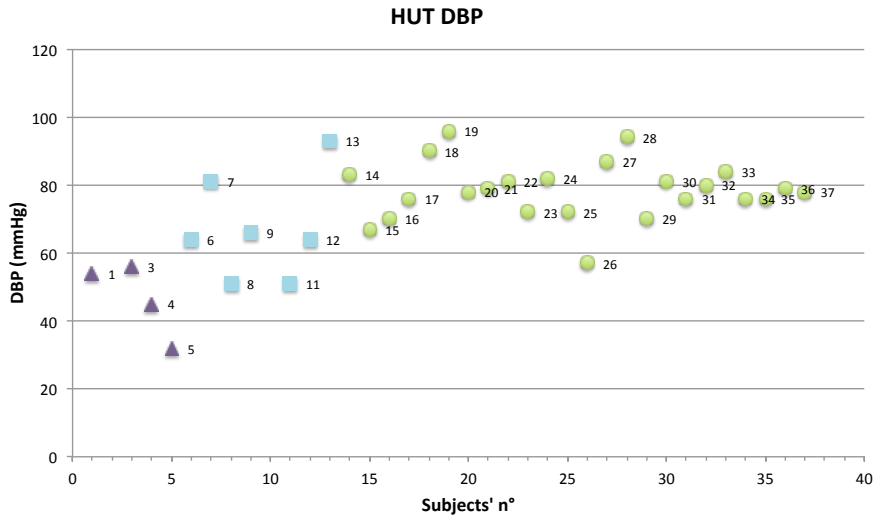


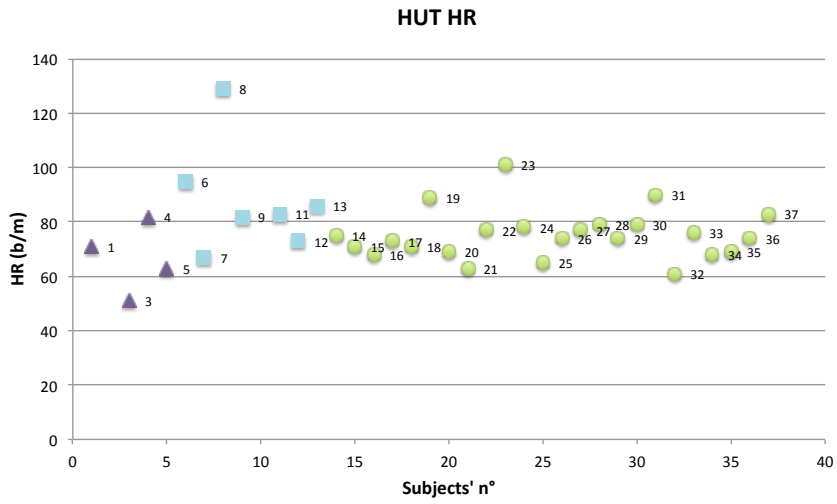
▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

APPENDIX: Section II.1

SBP, DBP and HR values during HUTT in SCI patients and controls



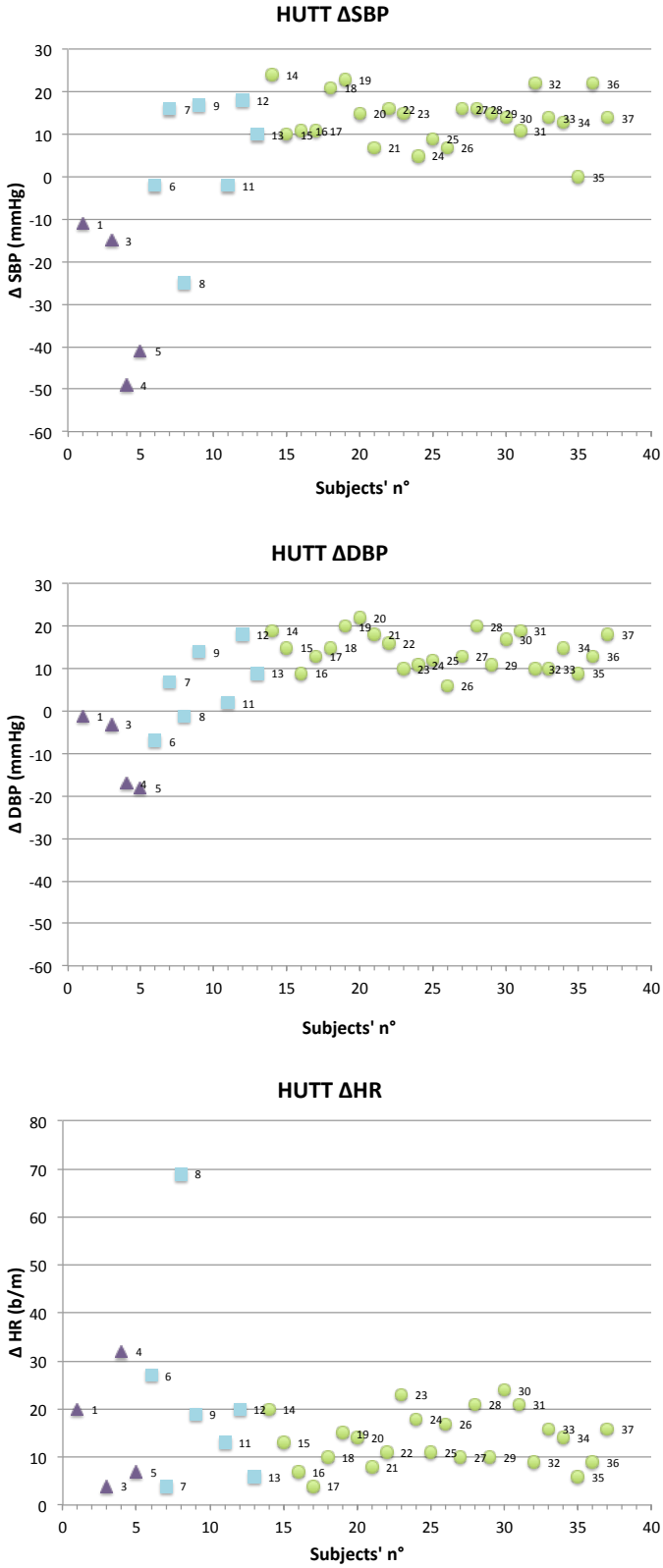




▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

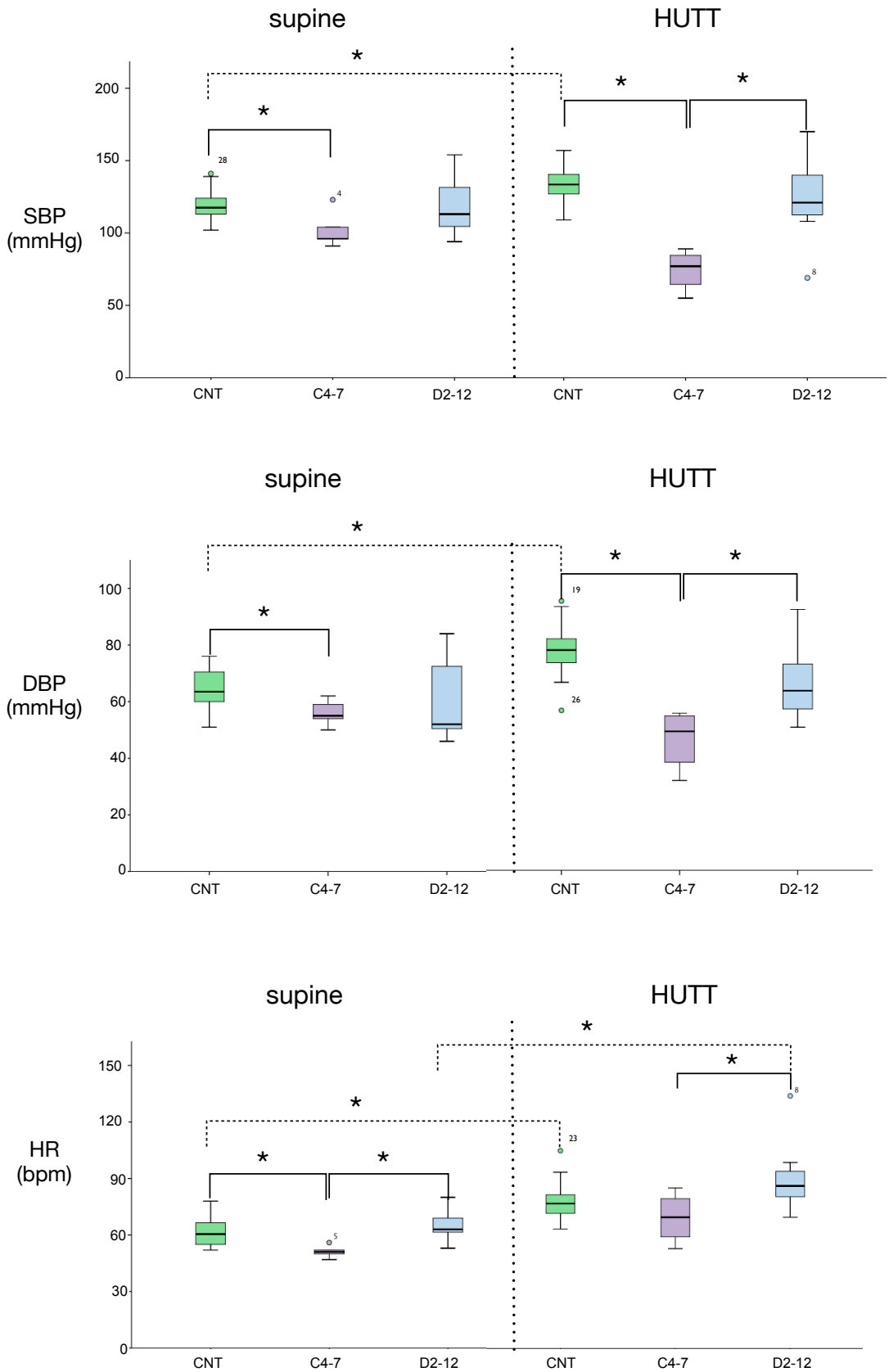
APPENDIX: Section II.1

Fig II.1.3: changes (Δ) in SBP, DBP and HR during HUTT in SCI patients and controls



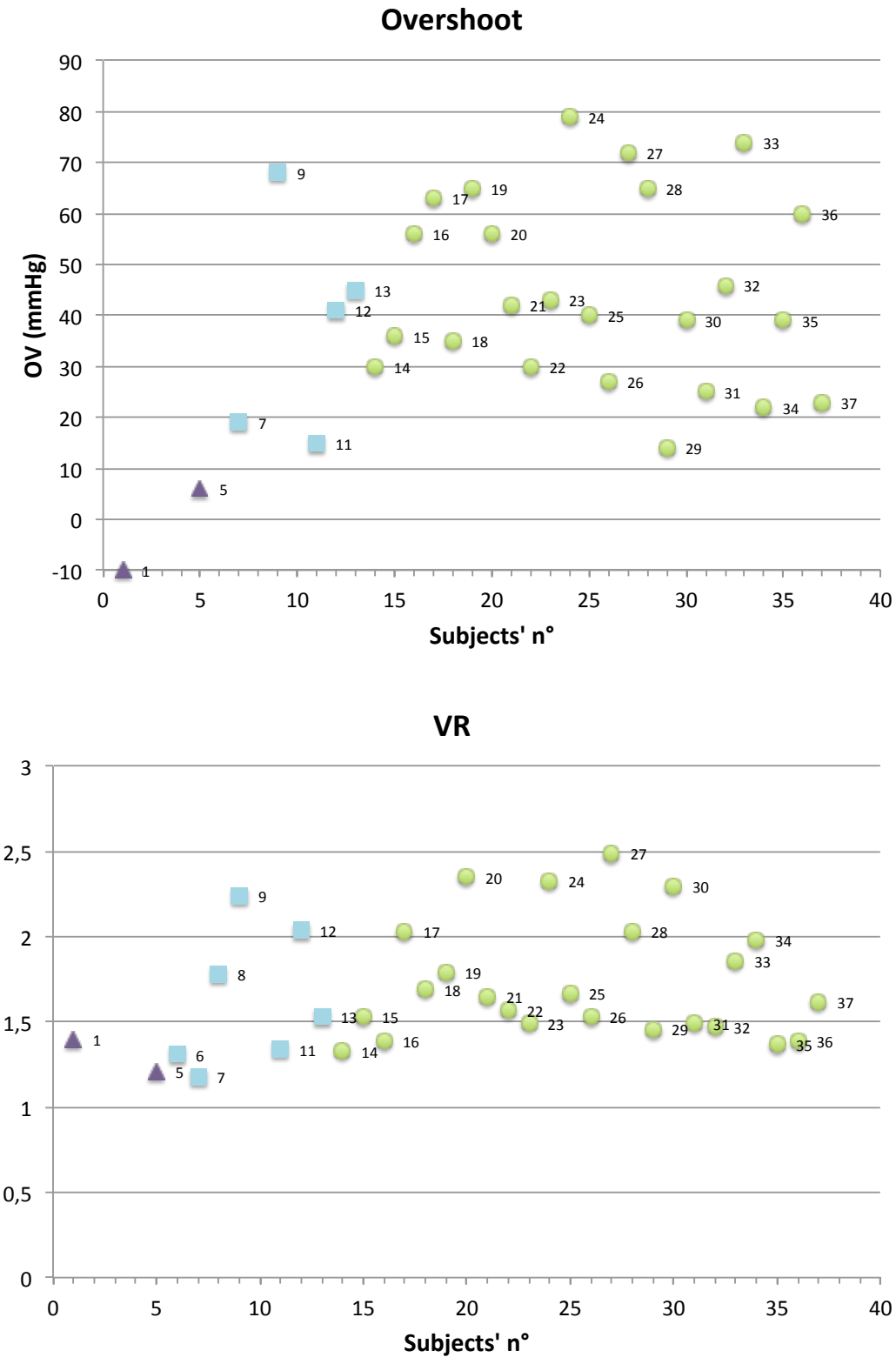
▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

Fig II.1.4: SBP, DBP and HR means±SD during supine rest and HUTT in SCI patients and controls



*=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

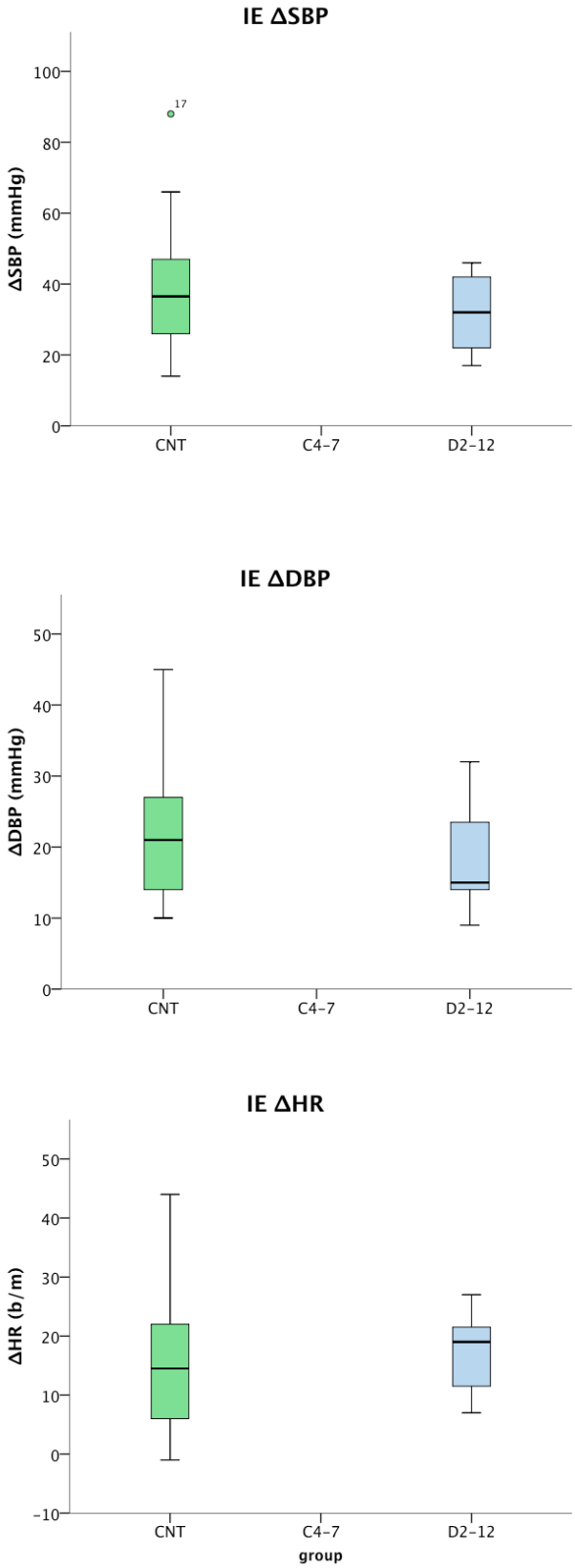
Fig II.1.5: overshoot (OV) and valsalva ratio (VR) in SCI patients and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

APPENDIX: Section II.1

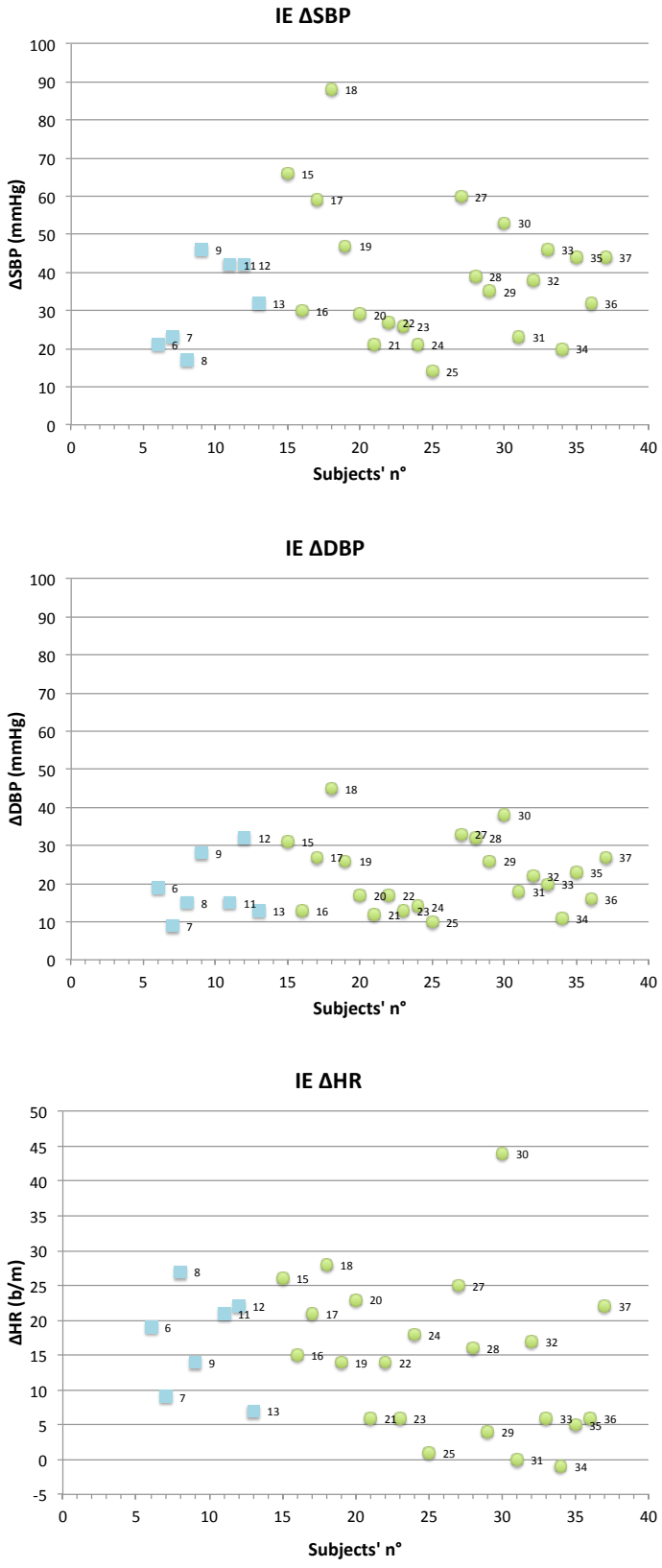
Fig II.1.6: mean ± SD changes (Δ) in SBP, DBP and HR during Isometric Exercise (IE) in SCI and controls



C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

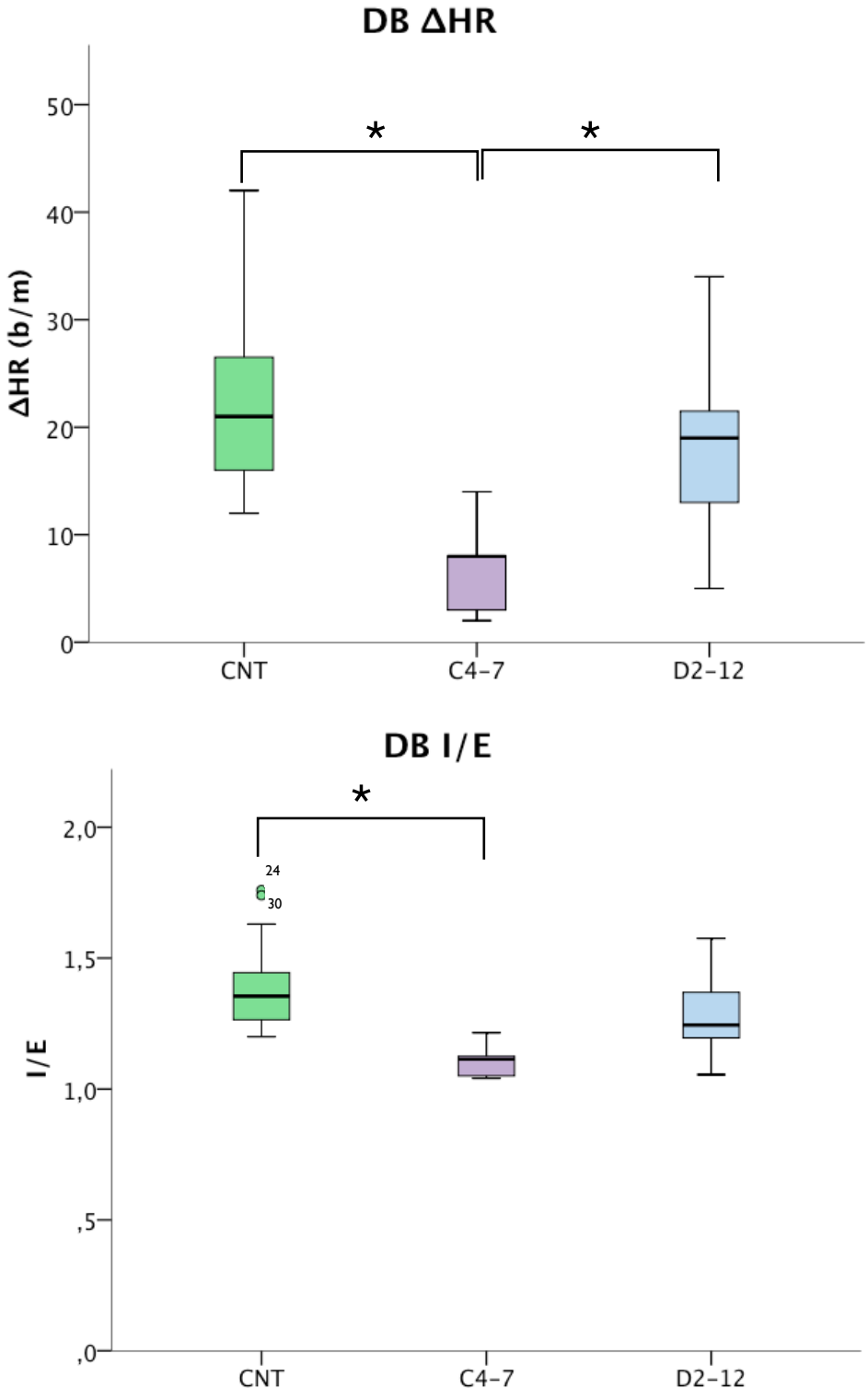
APPENDIX: Section II.1

Fig II.1.7: changes (Δ) in SBP, DBP and HR during Isometric Exercise (IE) in SCI and controls



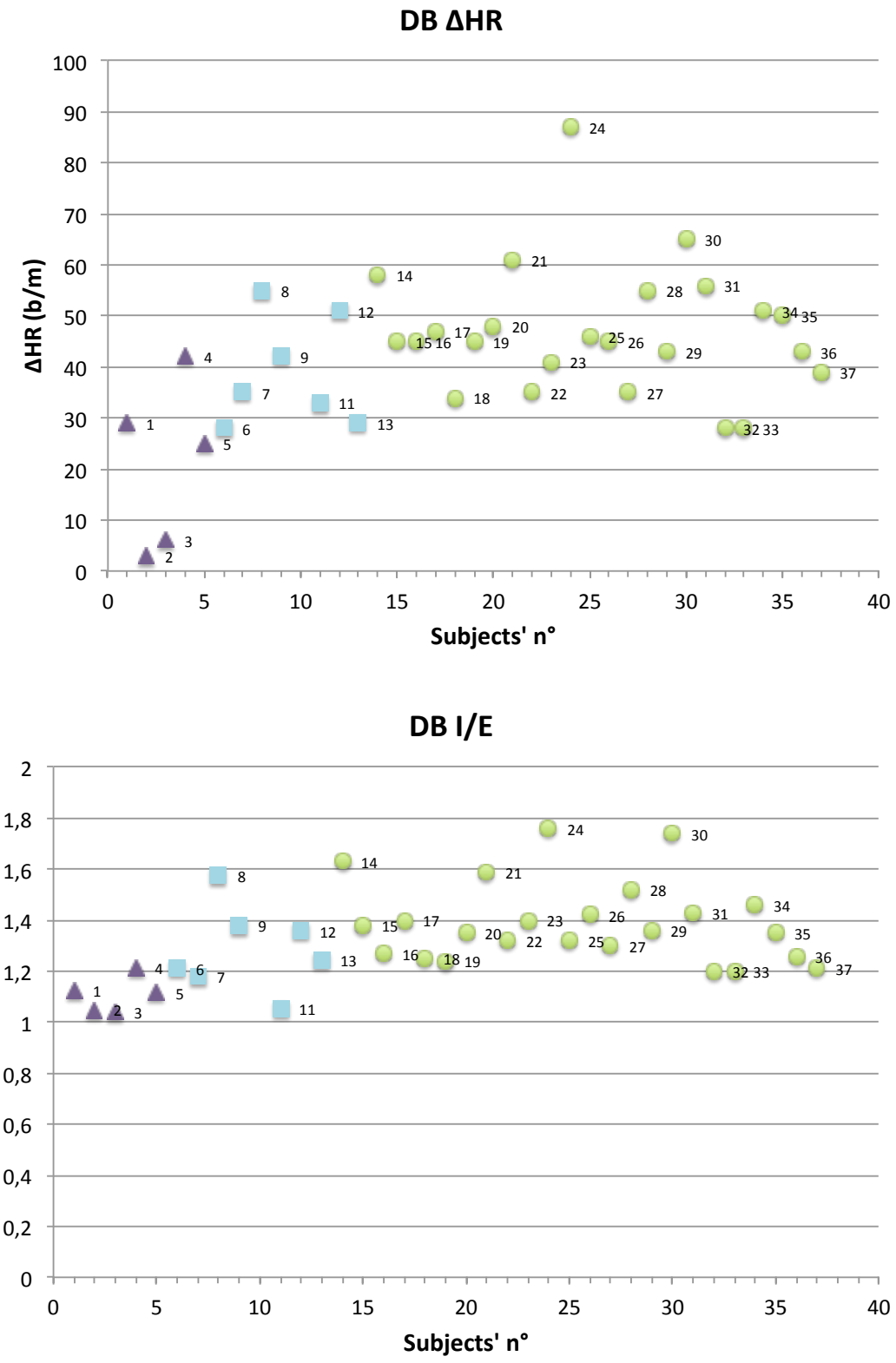
▲C4-7 ■D2-12 ●CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

Fig II.1.8: mean ± SD changes (Δ) in HR and I/E during Deep Breathing (DB) in SCI and controls



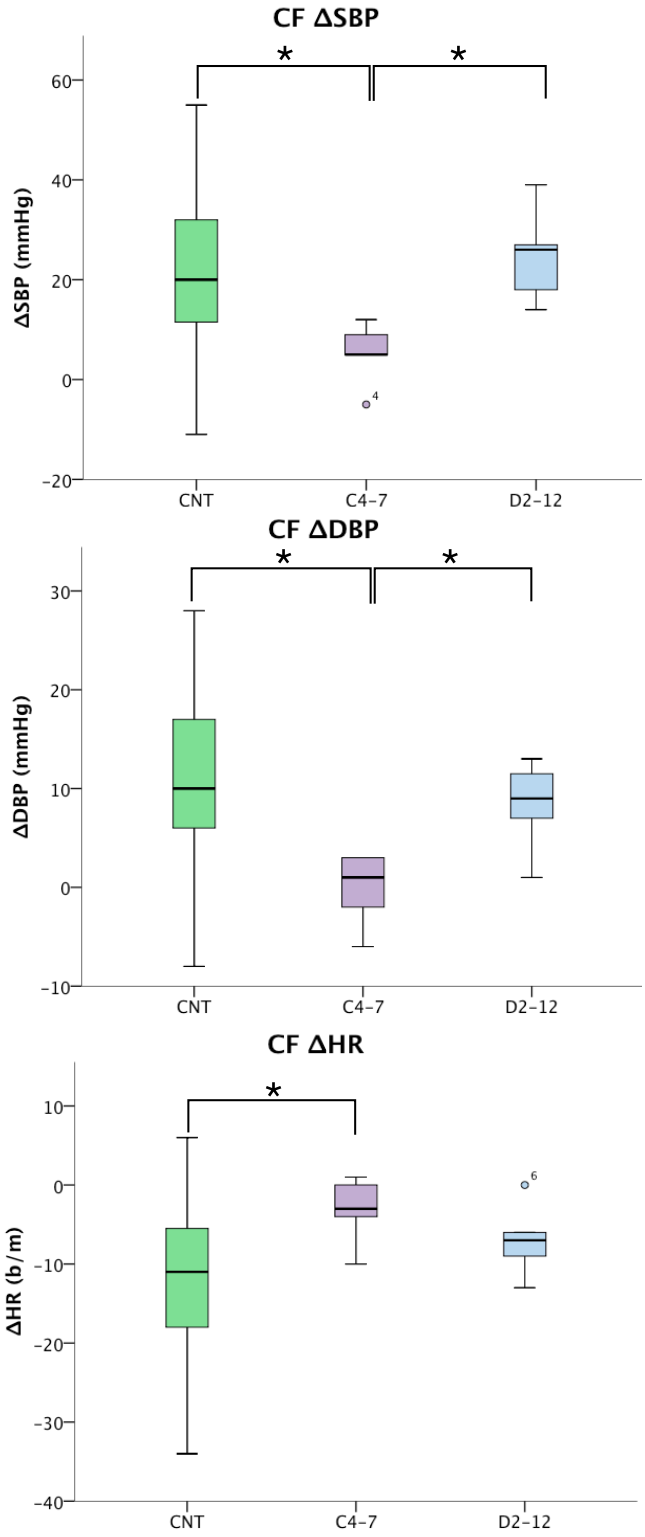
⋆=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; I/E: inspiratory/expiratory ratio. SCI: spinal cord injury.

Fig II.1.9: changes (Δ) in HR and I/E ratio during Deep Breathing (DB) in SCI and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; I/E: inspiratory/expiratory ratio; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

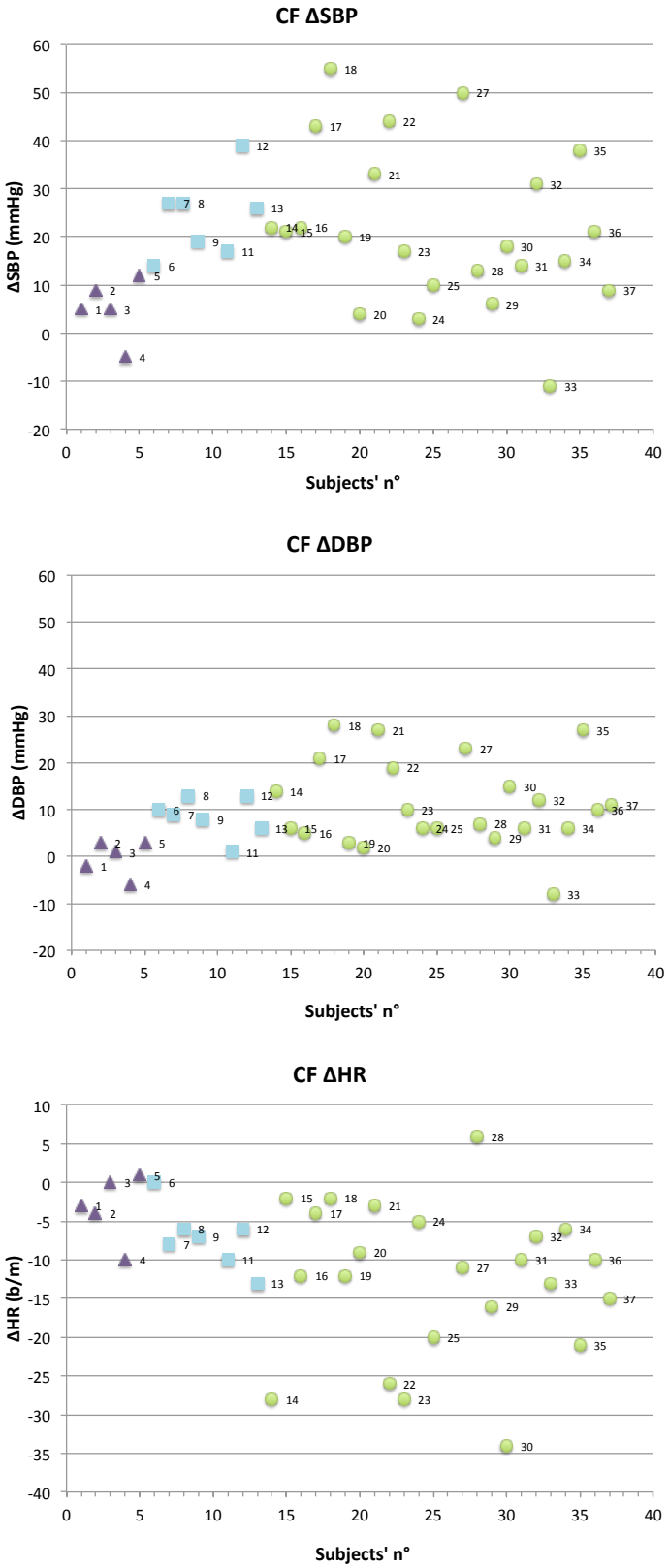
Fig II.1.10: mean ± SD changes (Δ) in SBP, DBP and HR during Cold Face (CF) in SCI and controls



⋆=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

APPENDIX: Section II.1

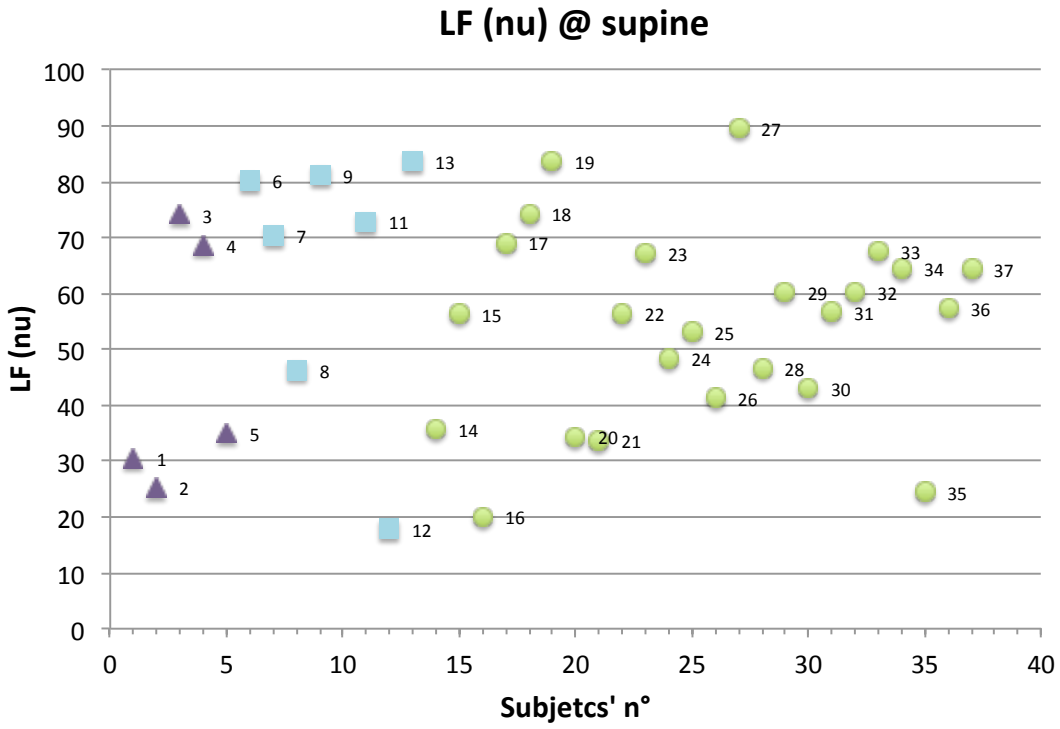
Fig II.1.11: changes (Δ) in SBP, DBP and HR during Cold Face (CF) in SCI and controls

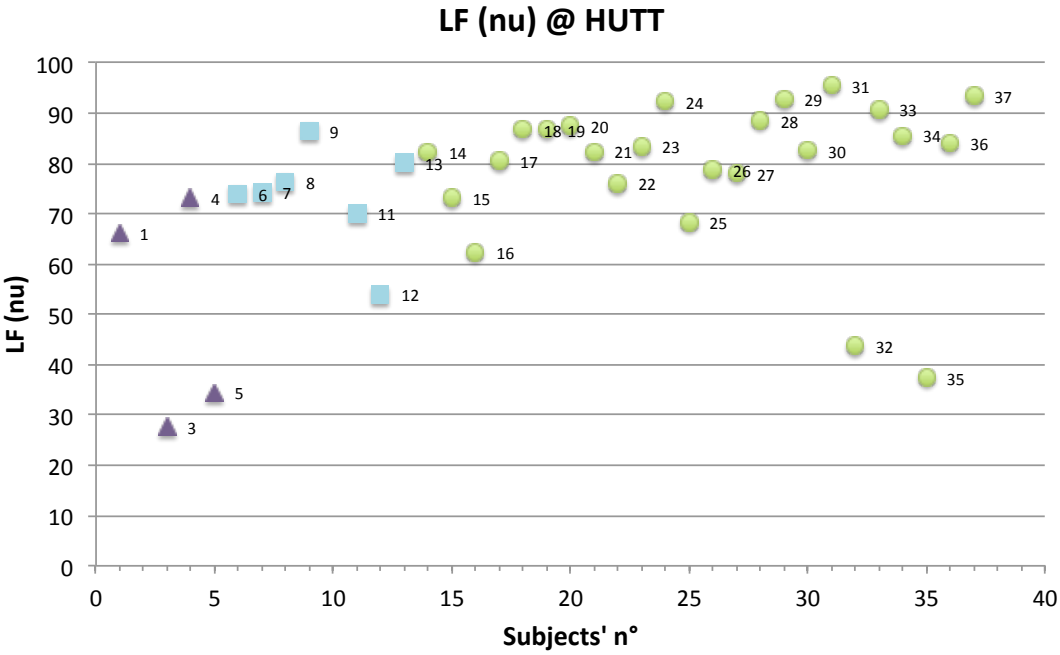


▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

APPENDIX: Section II.1

Low frequency component (LF nu) of heart rate variability during supine rest and HUTT

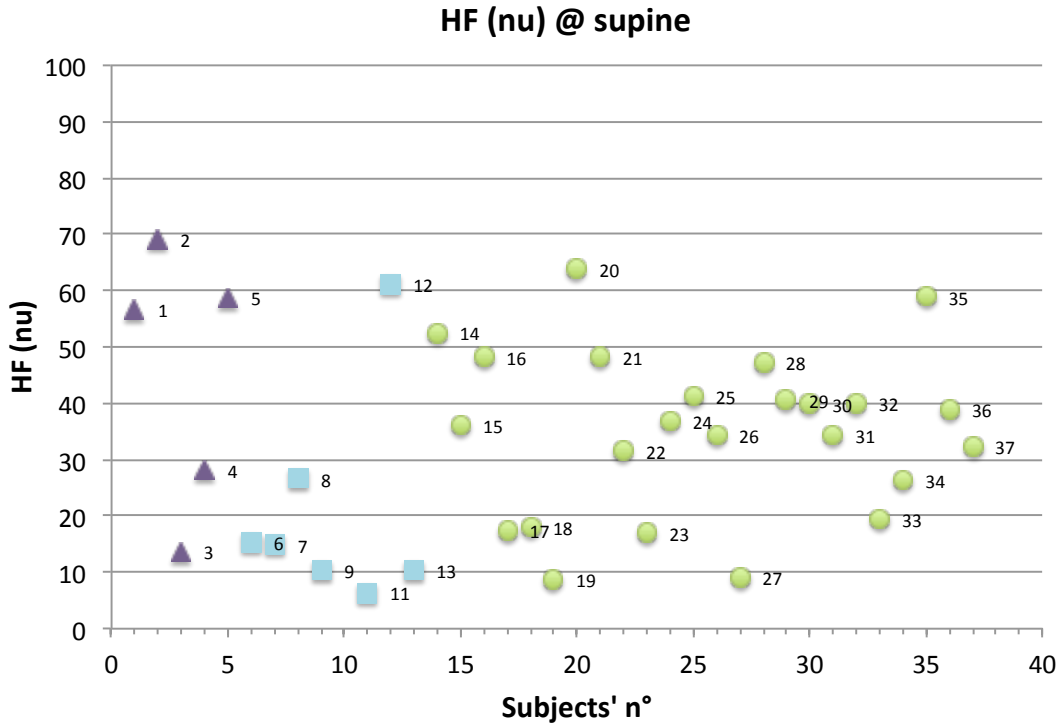


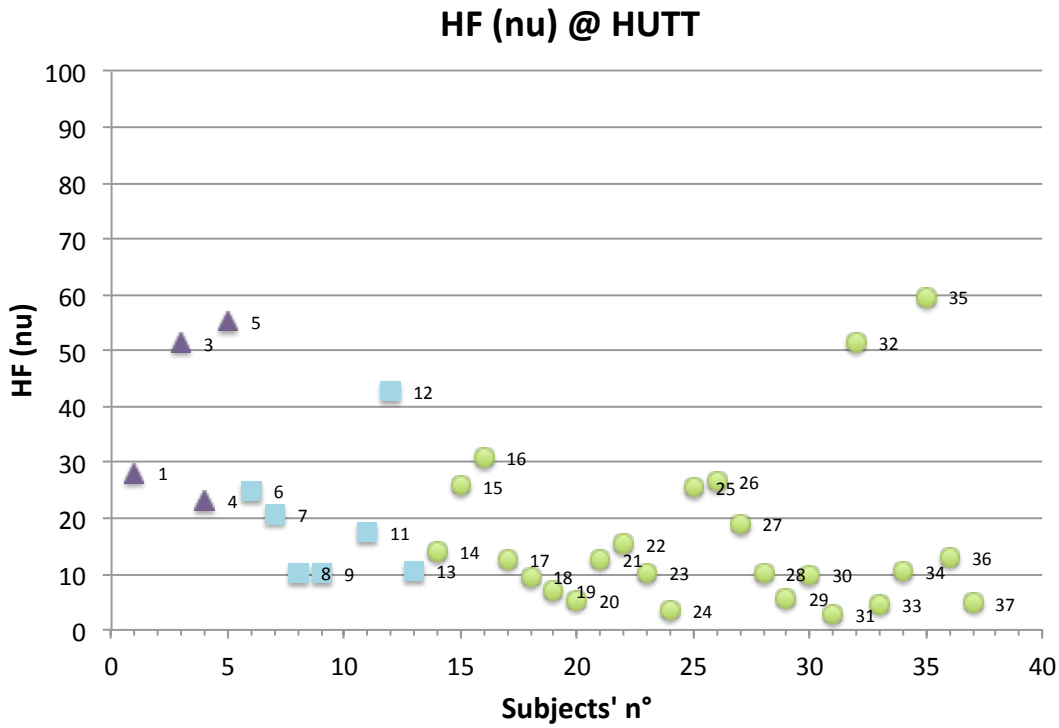


▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

APPENDIX: Section II.1

High frequency component (HF nu) of heart rate variability during supine rest and HUTT

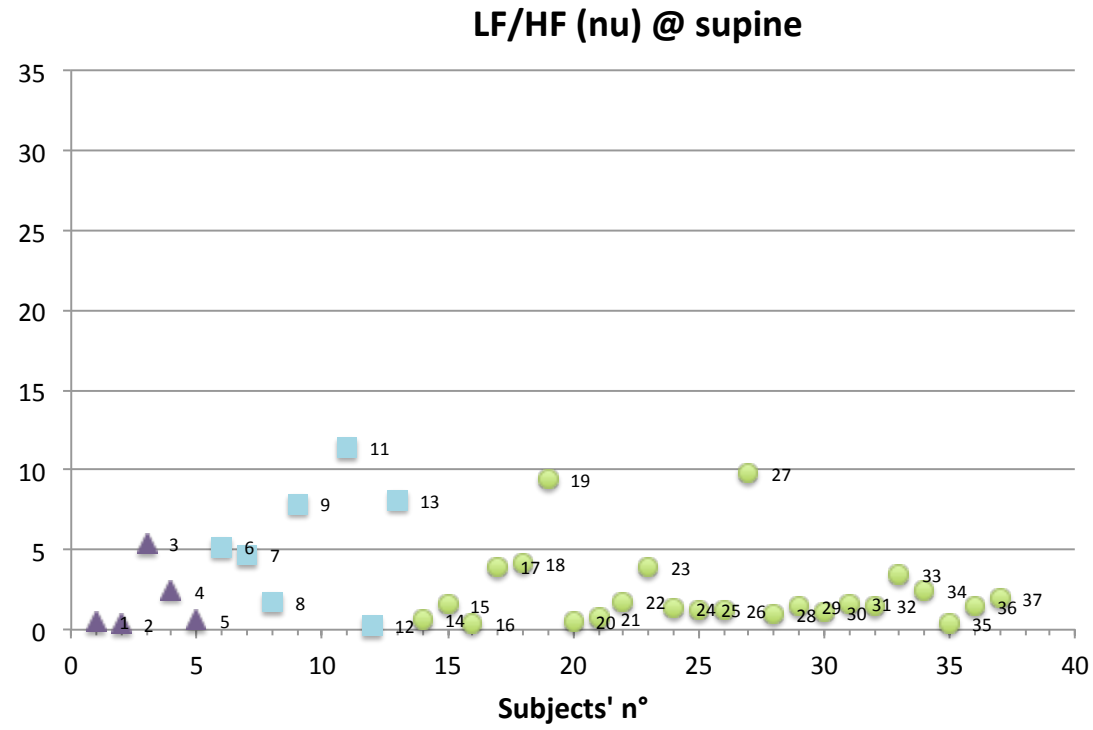


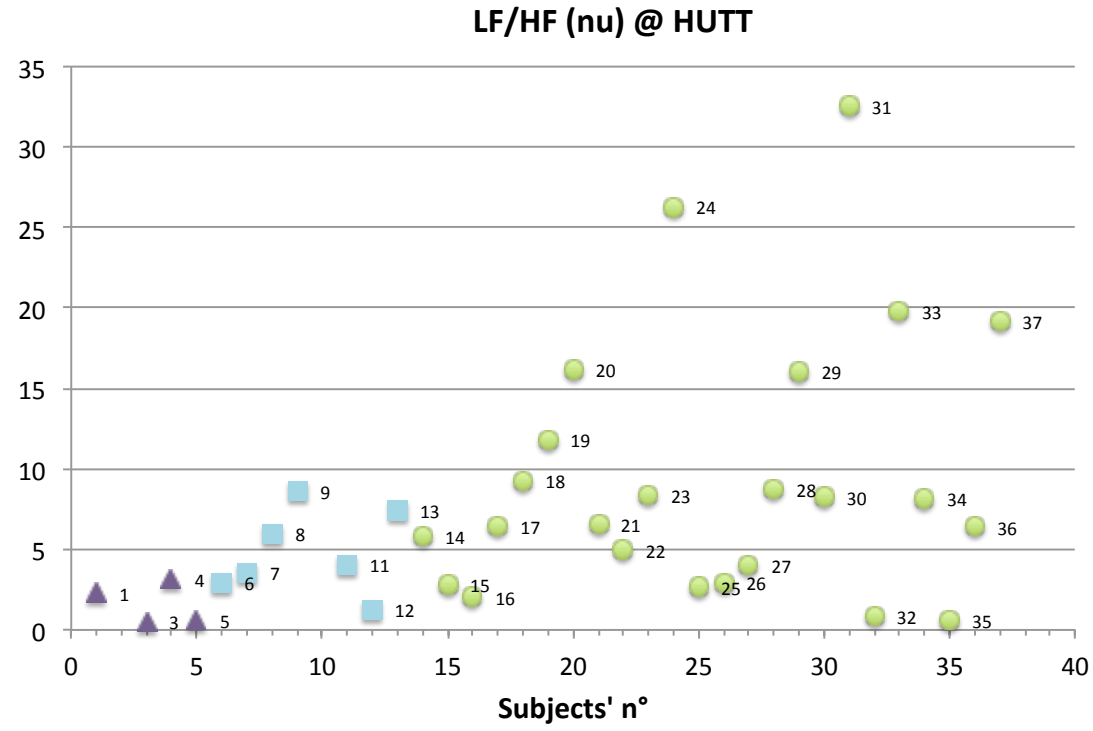


▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

APPENDIX: Section II.1

LF/HF ratio during supine rest and HUTT

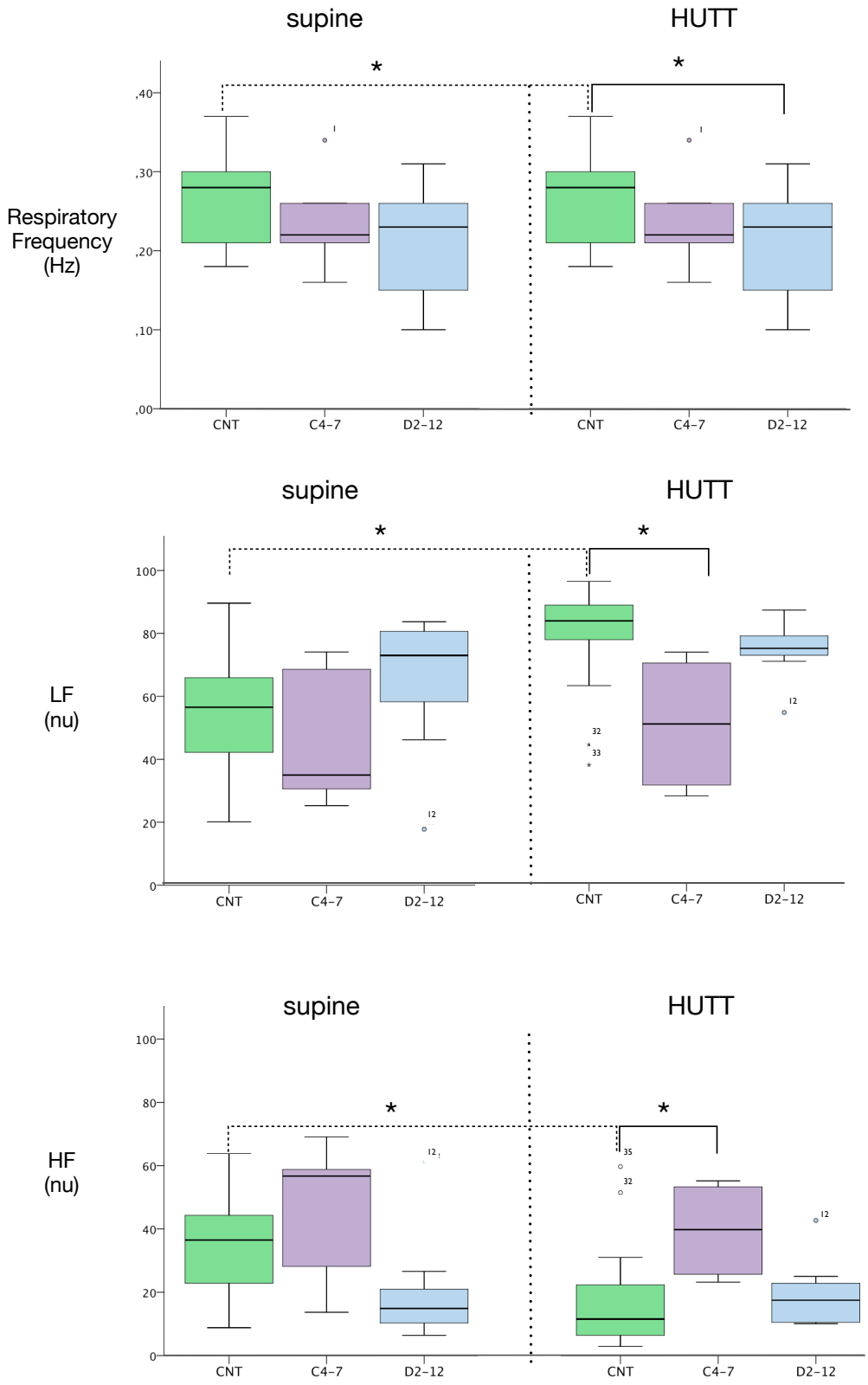




▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

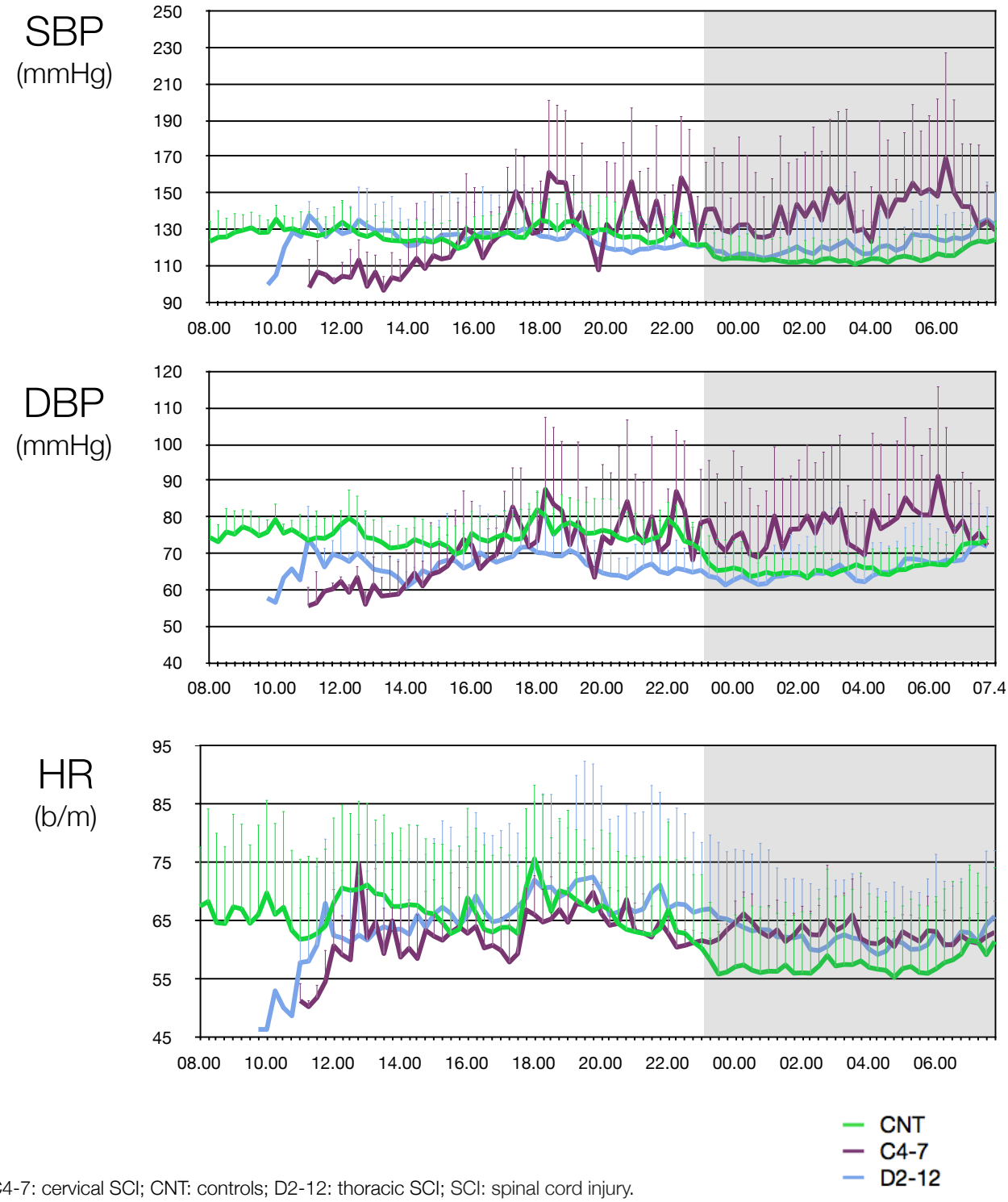
APPENDIX: Section II.1

Fig II.1.11: respiratory frequency, LF (nu) and HF (nu) during rest and HUTT in SCI and controls



*=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

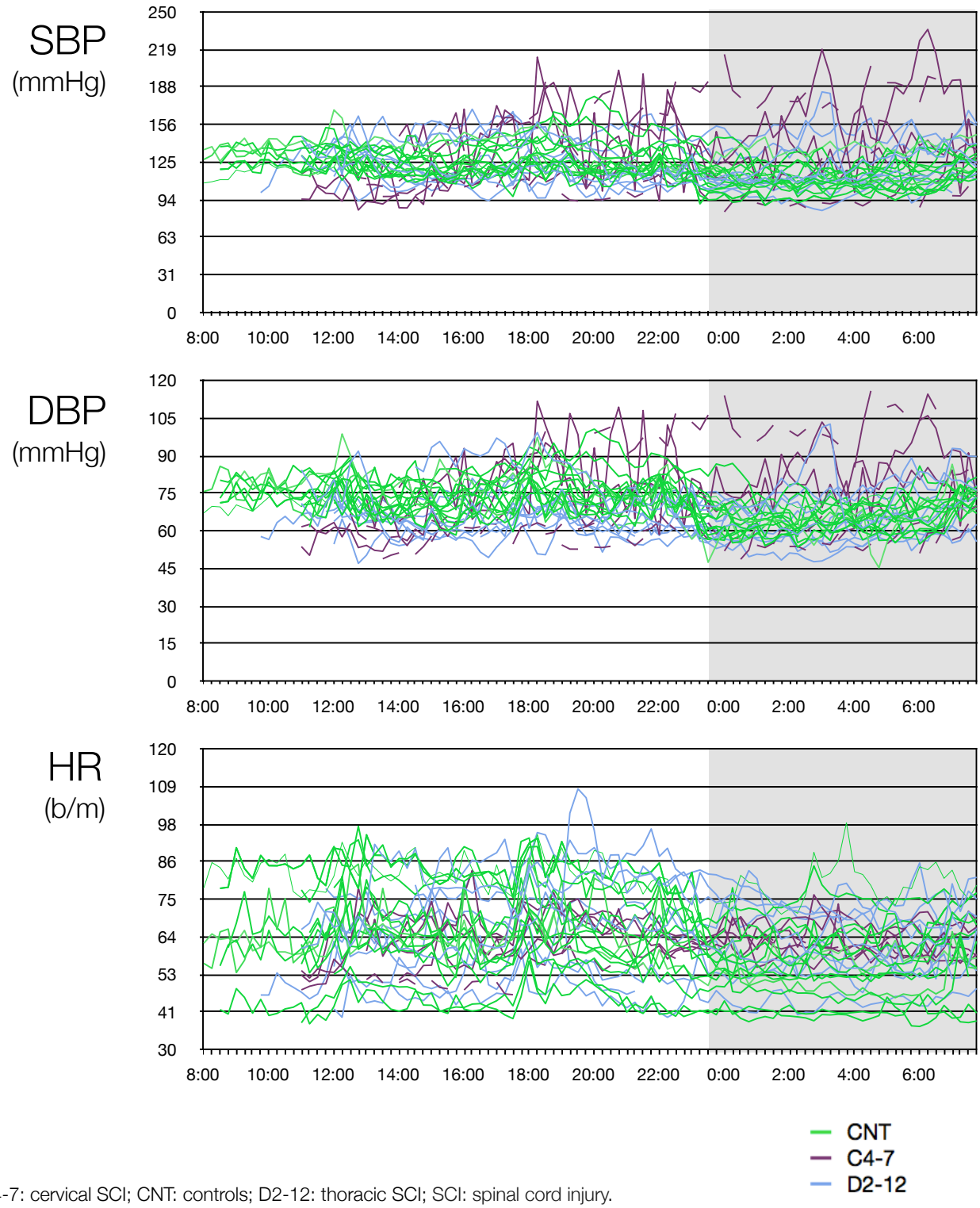
Fig II.2.2: 24-h pattern of SBP, DBP and HR in SCI patients and controls



C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

APPENDIX: Section II.2

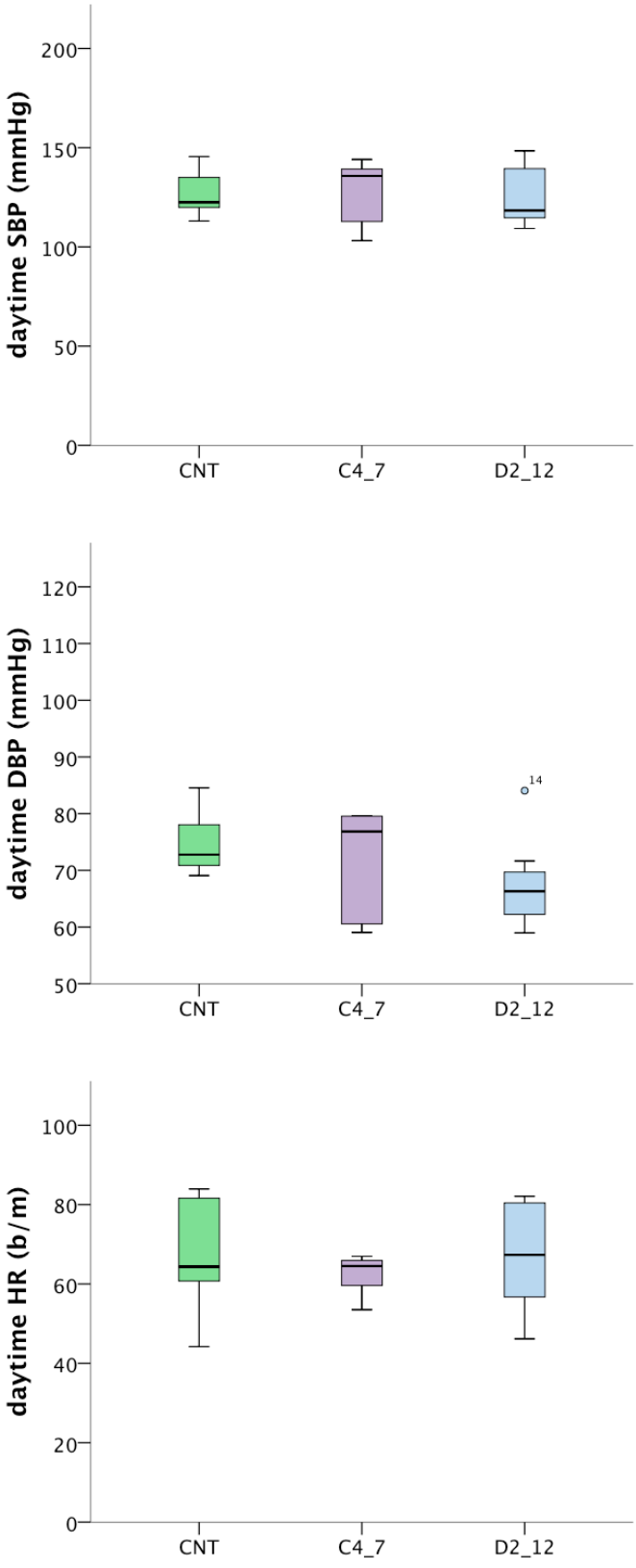
24-h pattern of SBP, DBP and HR in each SCI patient and control



C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

APPENDIX: Section II.2

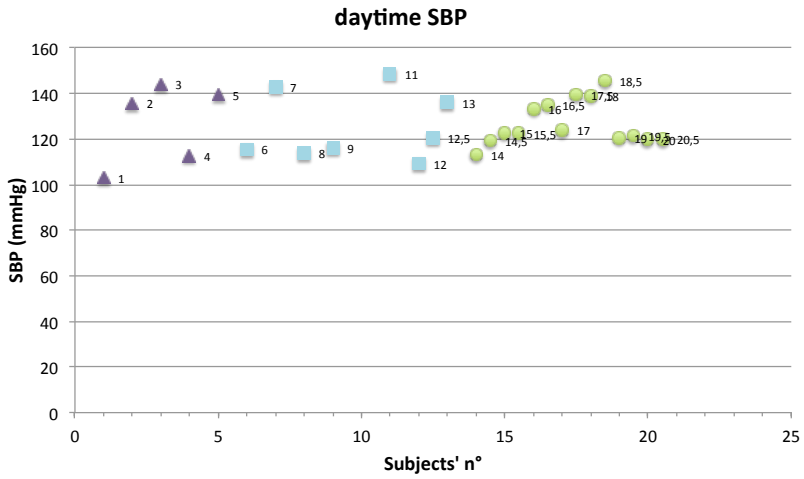
Daytime mean SBP, DBP and HR in SCI patients and controls

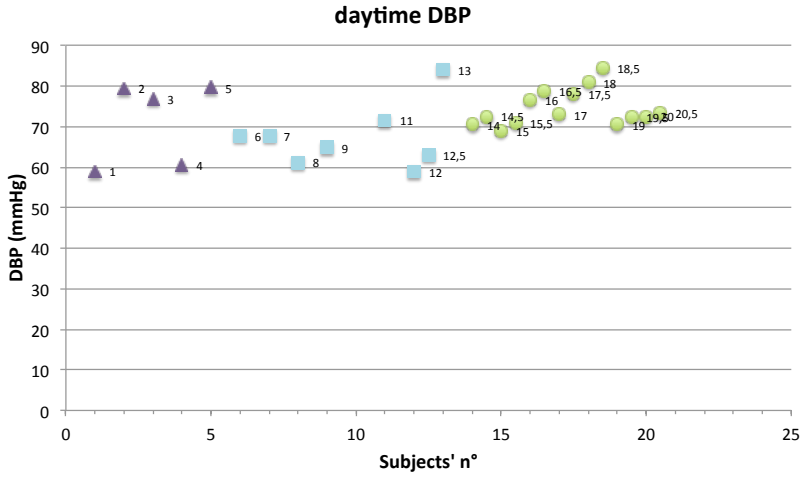


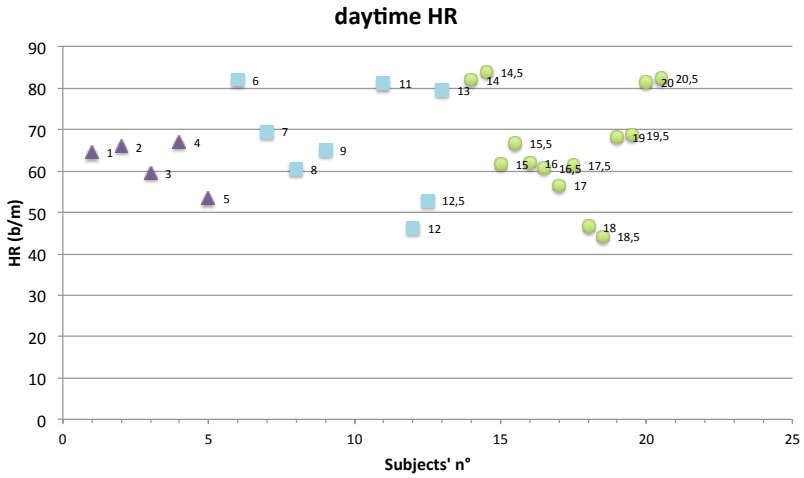
C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury

APPENDIX: Section II.2

Daytime SBP, DBP and HR in SCI patients and controls



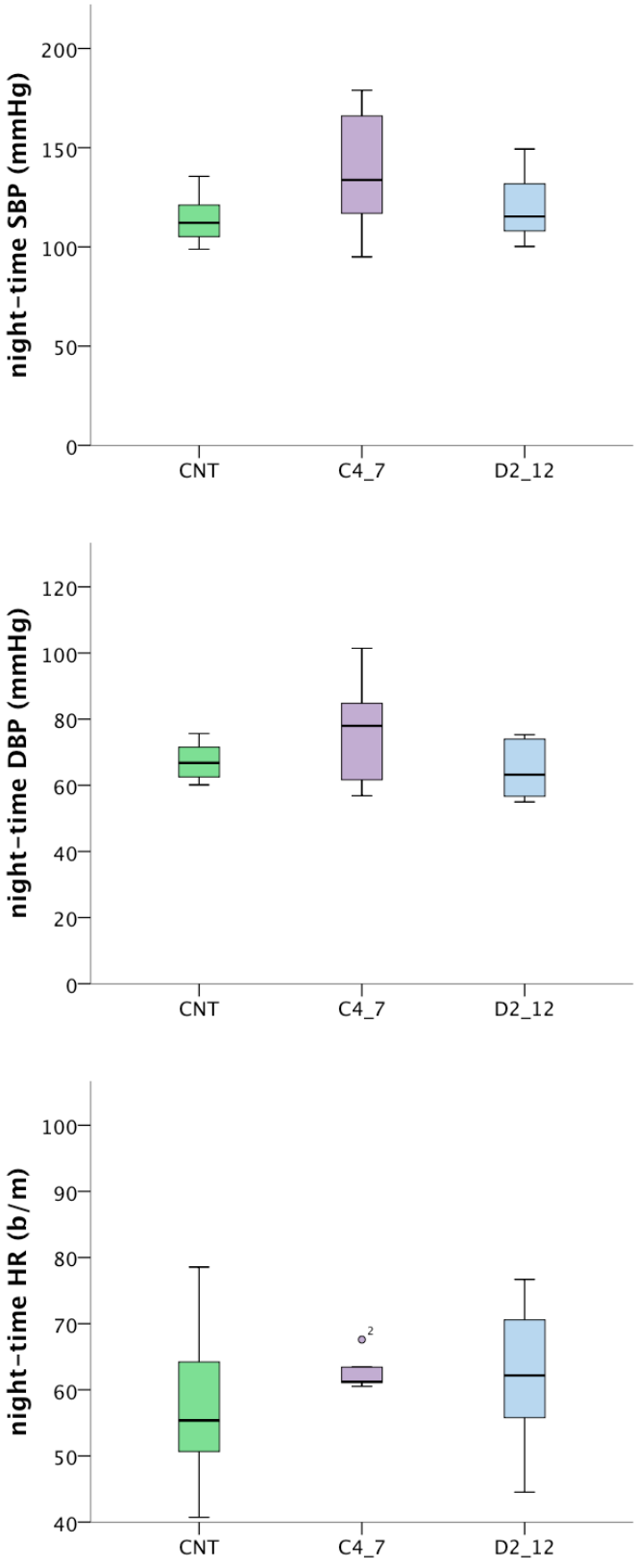




▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

APPENDIX: Section II.2

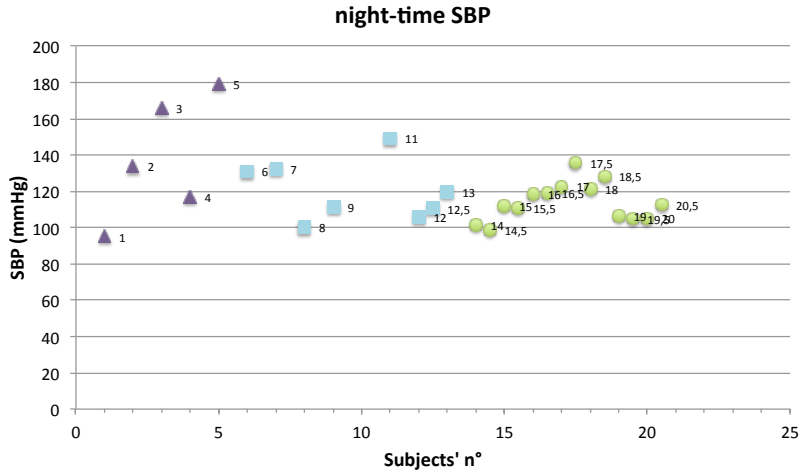
Night-time mean SBP, DBP and HR in SCI patients and controls

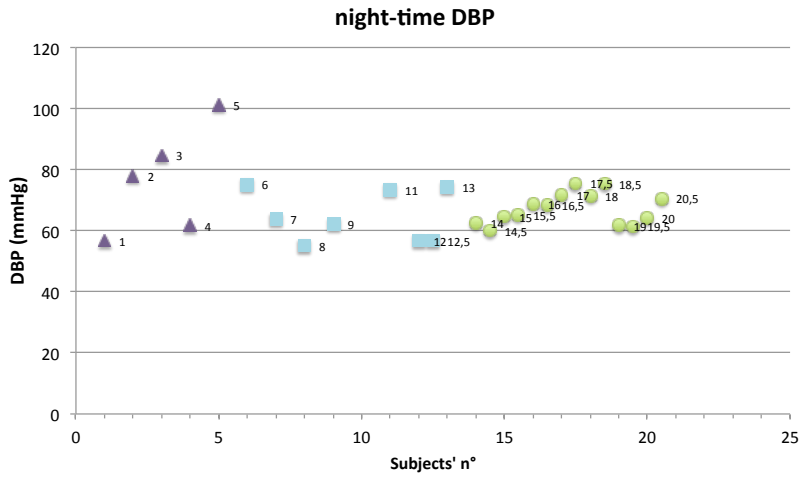


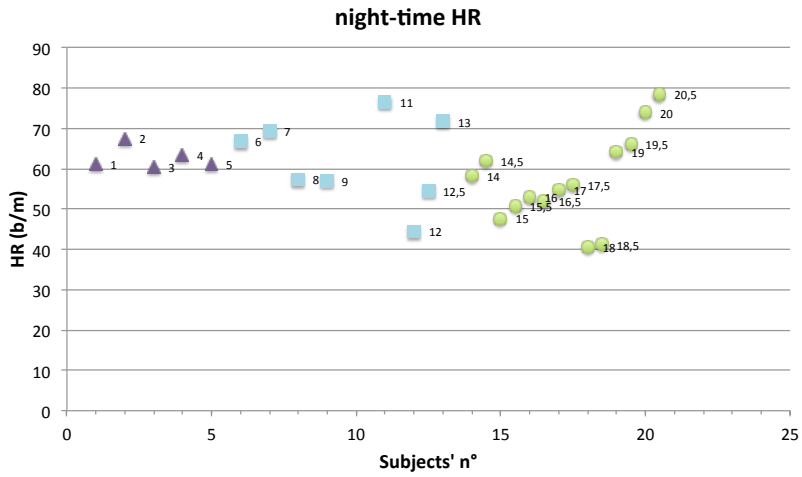
C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

APPENDIX: Section II.2

Night-time SBP, DBP and HR in SCI patients and controls

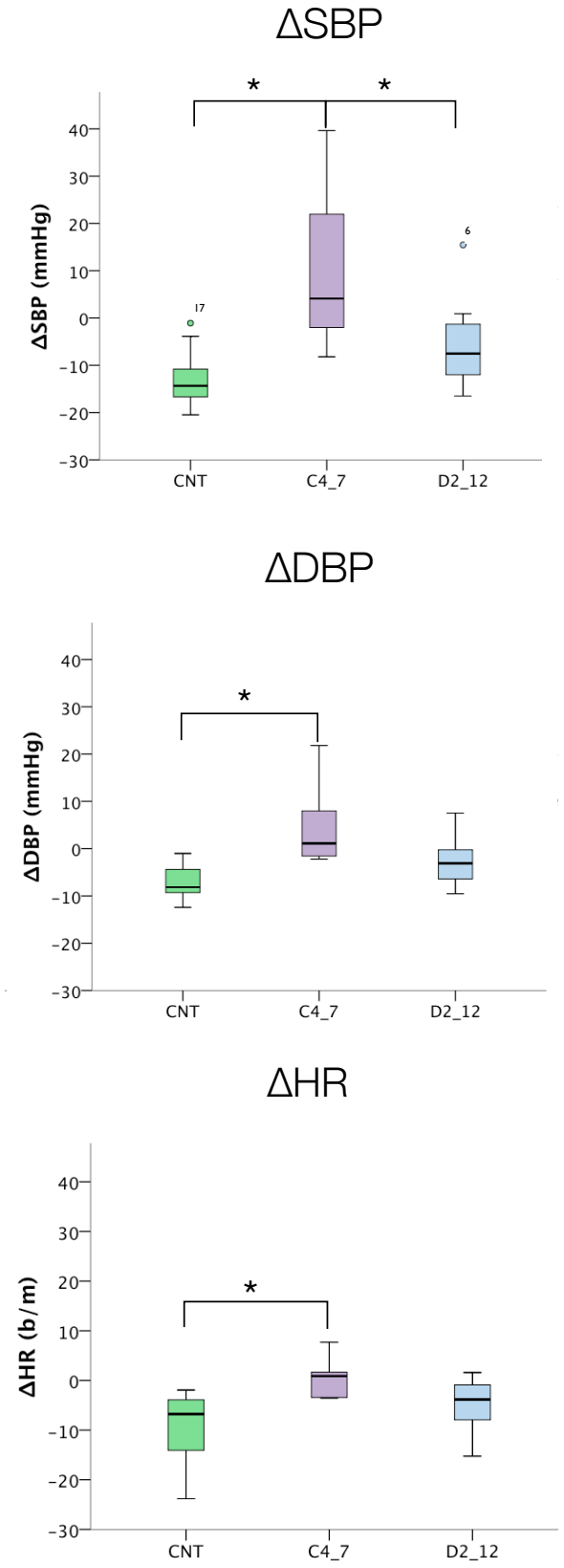






▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

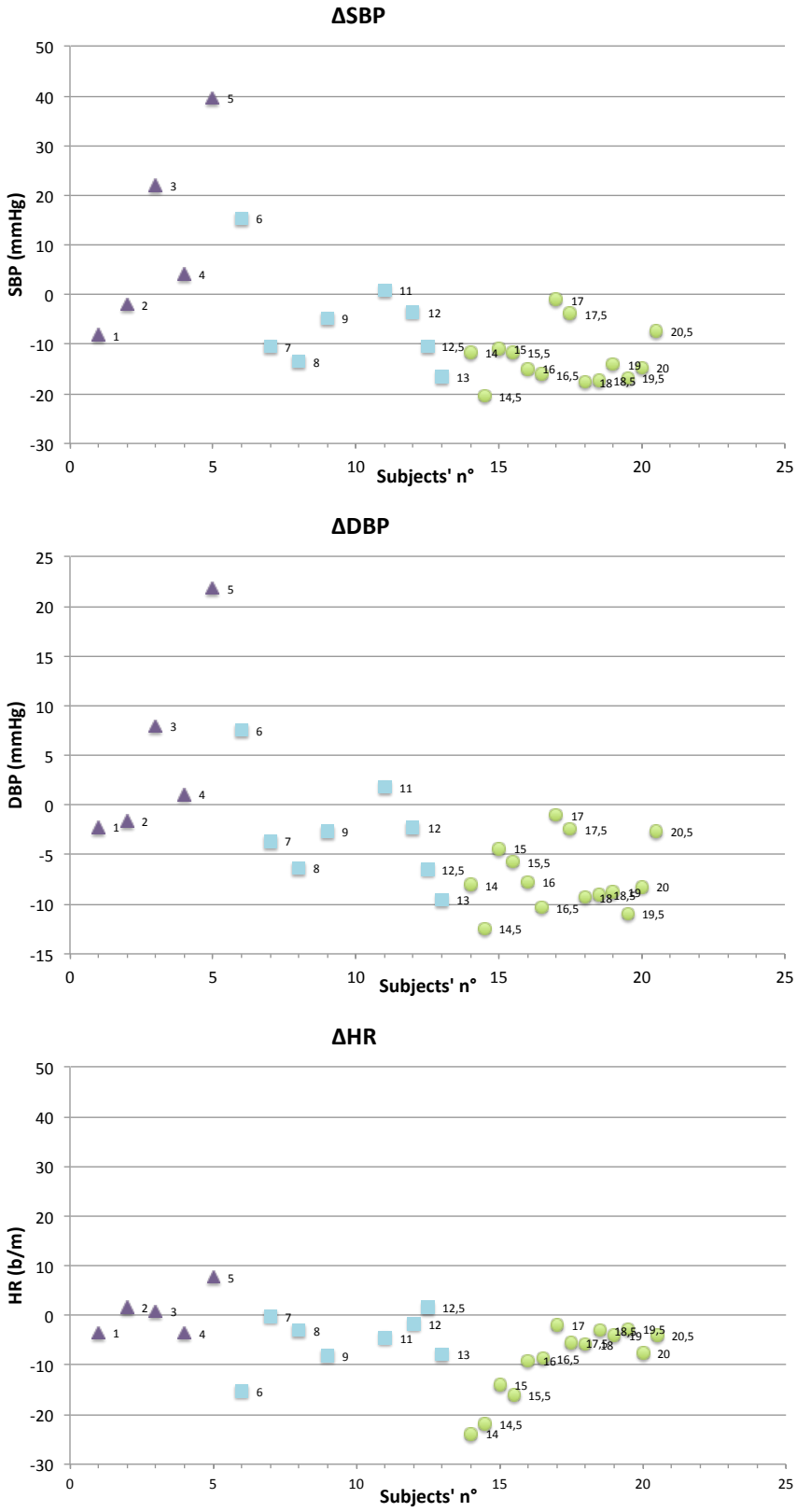
Fig II.2.3: mean day-night changes (Δ) in SBP, DBP and HR in SCI patients and controls



*=p<0,05. C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury

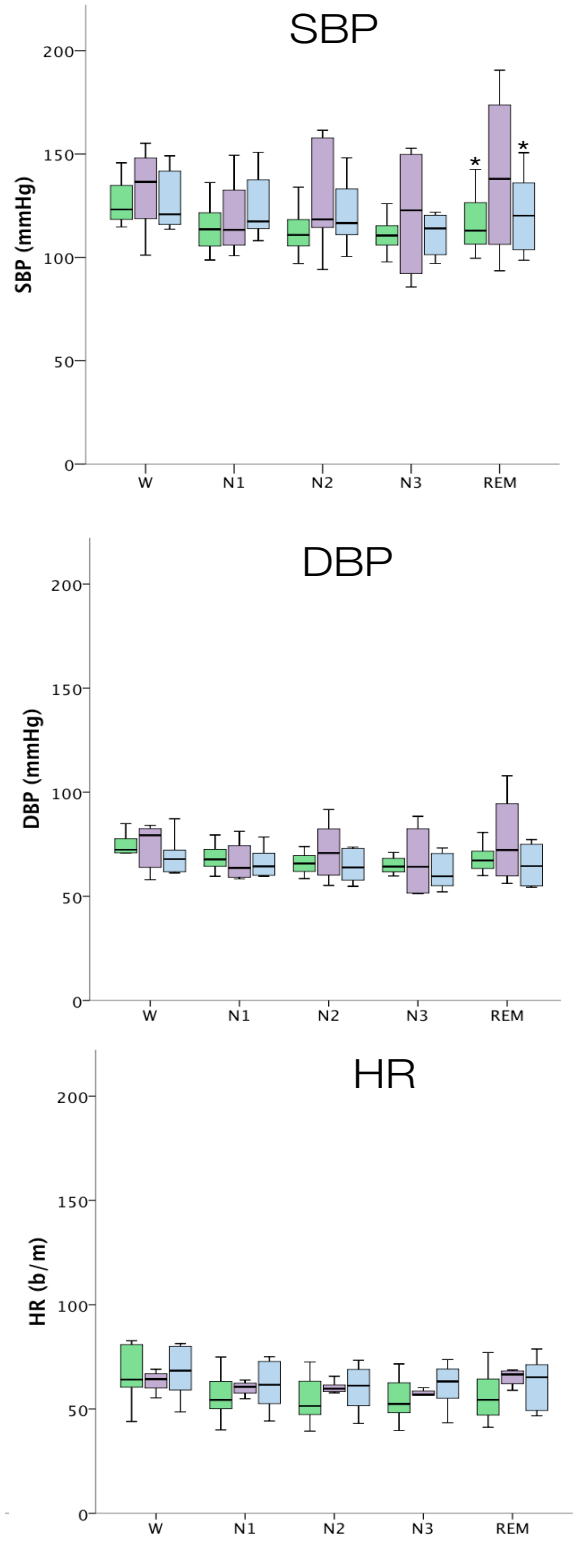
APPENDIX: Section II.2

Fig II.2.4: day-night changes (Δ) in SBP, DBP and HR in SCI patients and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI. Numbers on axis of abscissas correspond to subjects' numbers.

Fig II.2.5: state dependent changes in SBP, DBP and HR in SCI and controls (between groups)



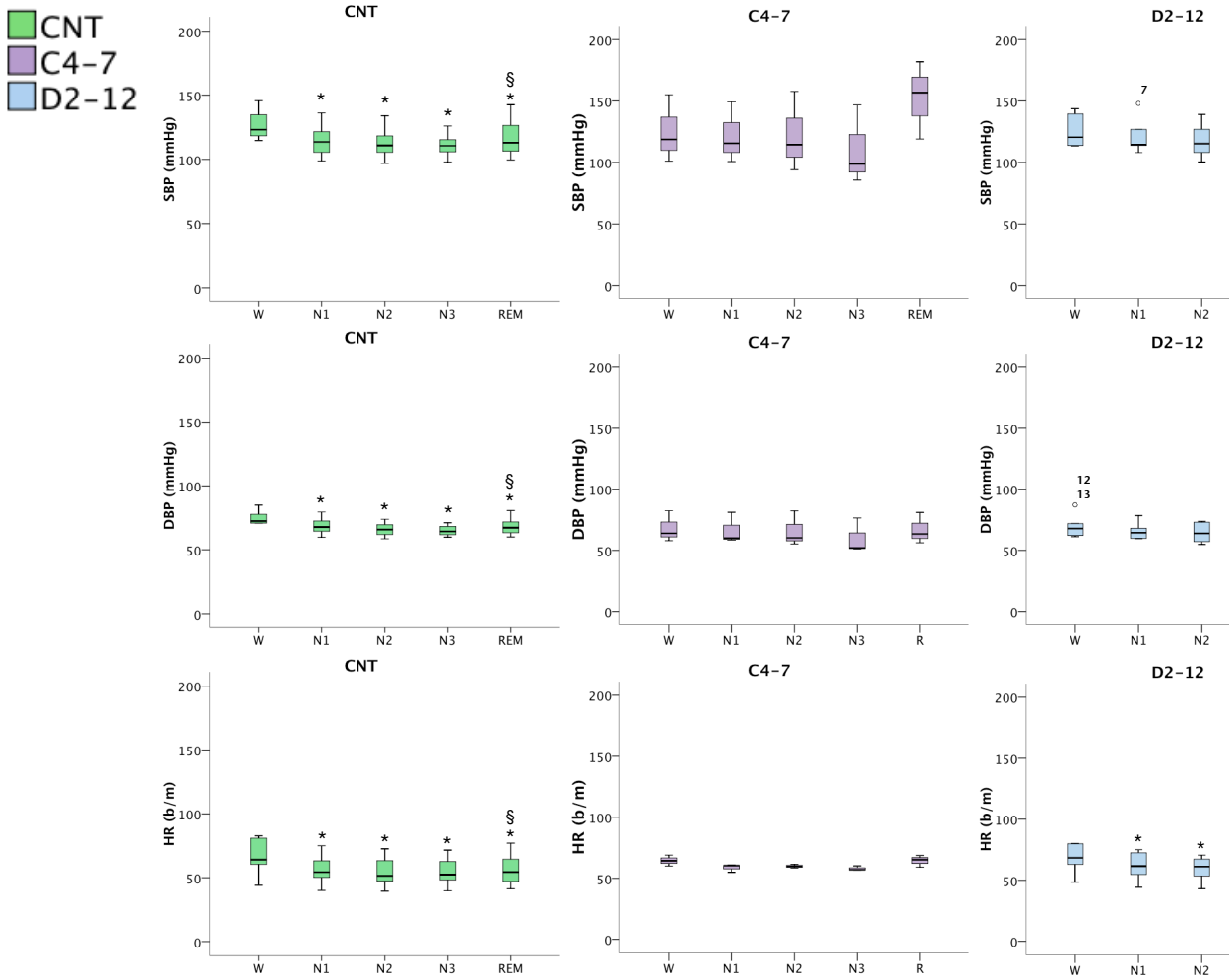
CNT
C4-7
D2-12

*=p<0,01 *vs*. cervical SCI.

C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

APPENDIX: Section II.2

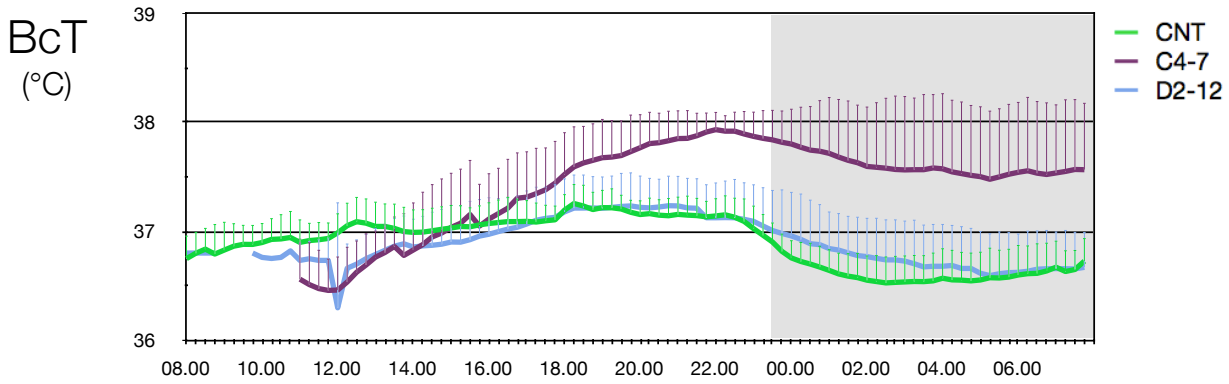
Fig II.2.6: state dependent changes in SBP, DBP and HR in SCI and controls (between groups)



*=*p*<0,05 *vs* within group wake (W) values; §=p<0,03 *vs* within group stage 3 (N3) NREM sleep values

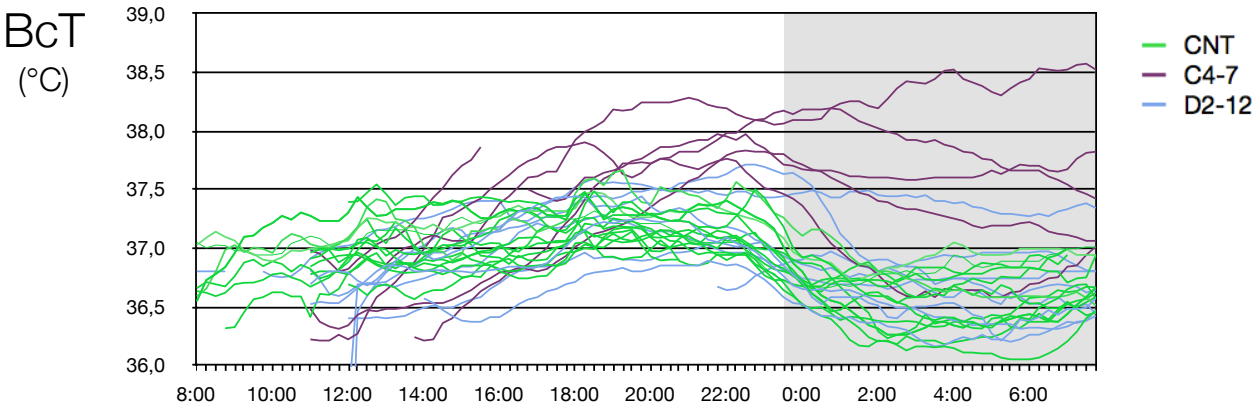
C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

Fig III.3: 24-h pattern of BcT in SCI patients and controls



BcT: body core temperature; C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

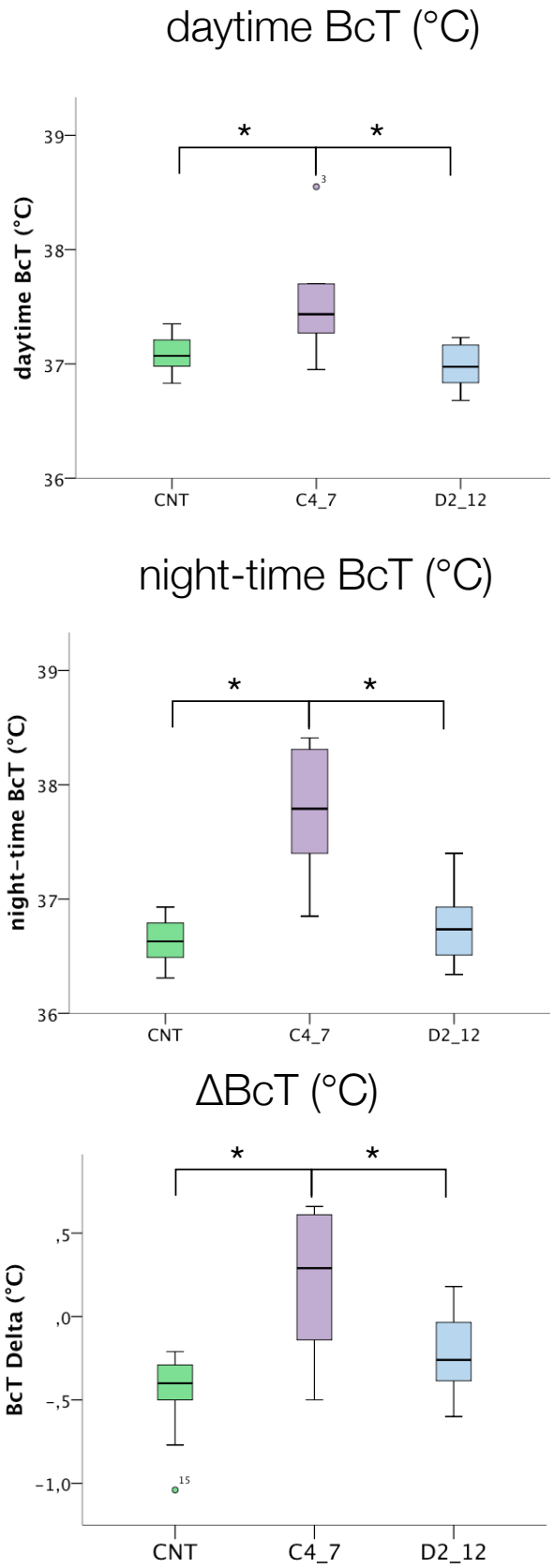
24-h pattern of BcT in each SCI patient and control



BcT: body core temperature; C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; SCI: spinal cord injury.

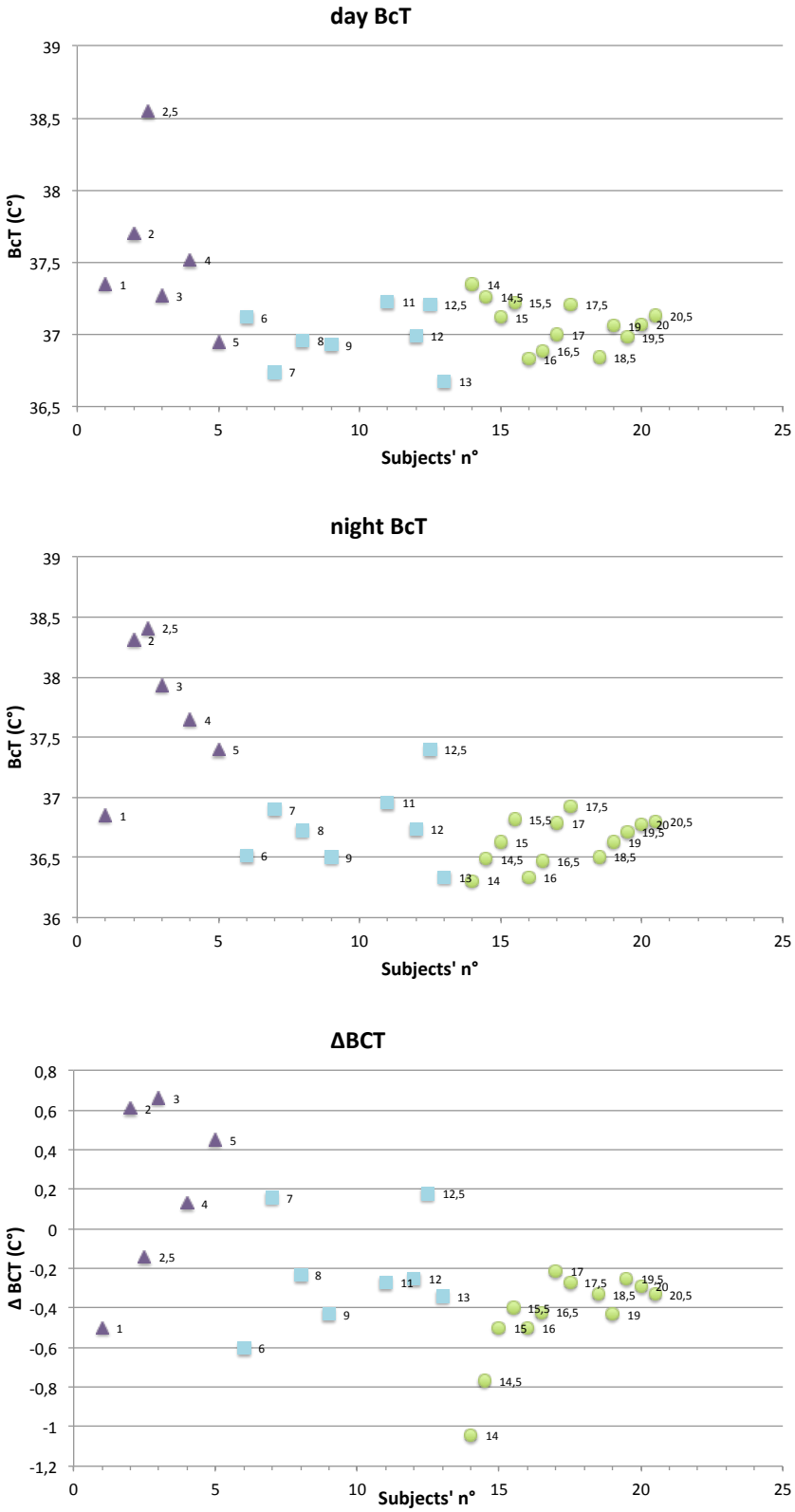
APPENDIX: Section III

Fig III.3: daytime BcT, night-time BcT and day-night changes (Δ) in BcT in SCI patients and controls



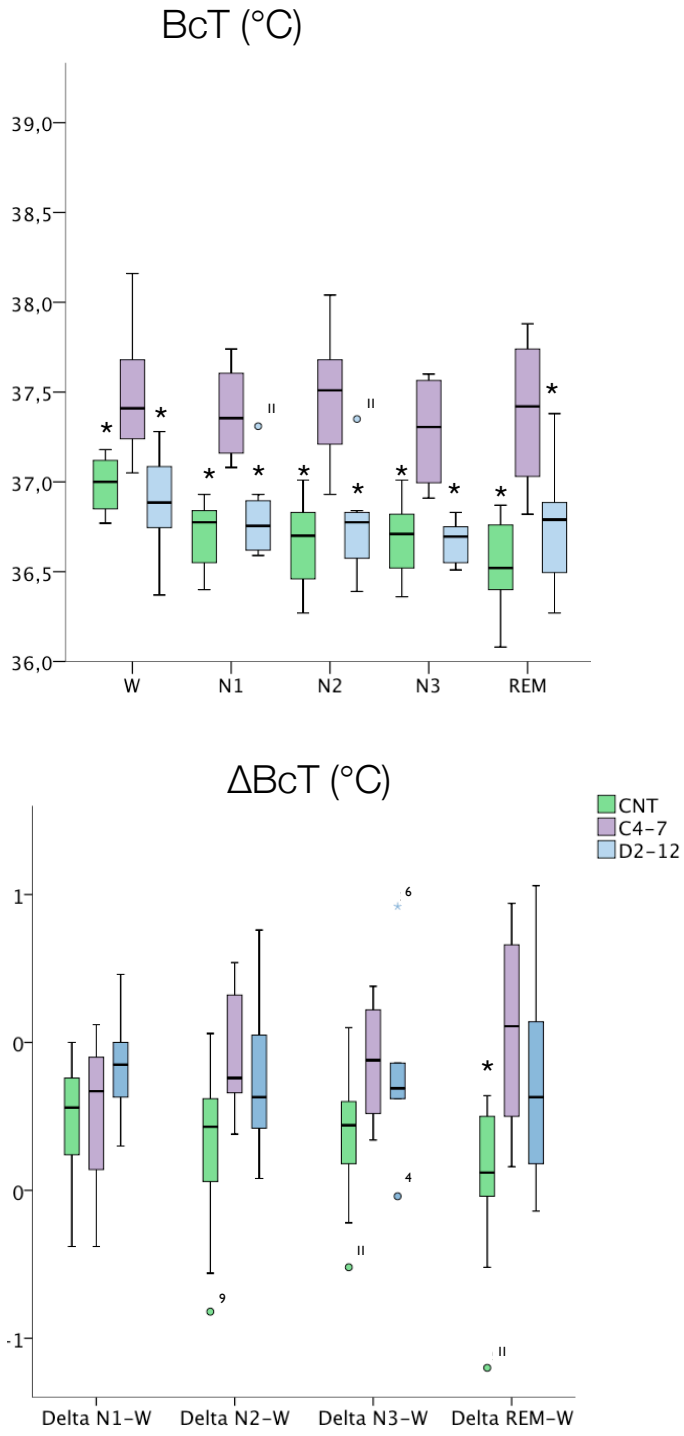
*=p<0,05. BcT: body core temperature; C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; Δ: day-night changes.

Fig III.5: daytime BcT, night-time BcT and day-night changes (Δ) in BcT in SCI patients and controls



▲ C4-7 ■ D2-12 ● CNT C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI;SCI: spinal cord injury. Numbers on axis of abscissas correspond to subjects' numbers.

Fig III.6: state dependent variation of BcT and BcT changes (Δ) with respect to wake in SCI patients and controls (between groups)

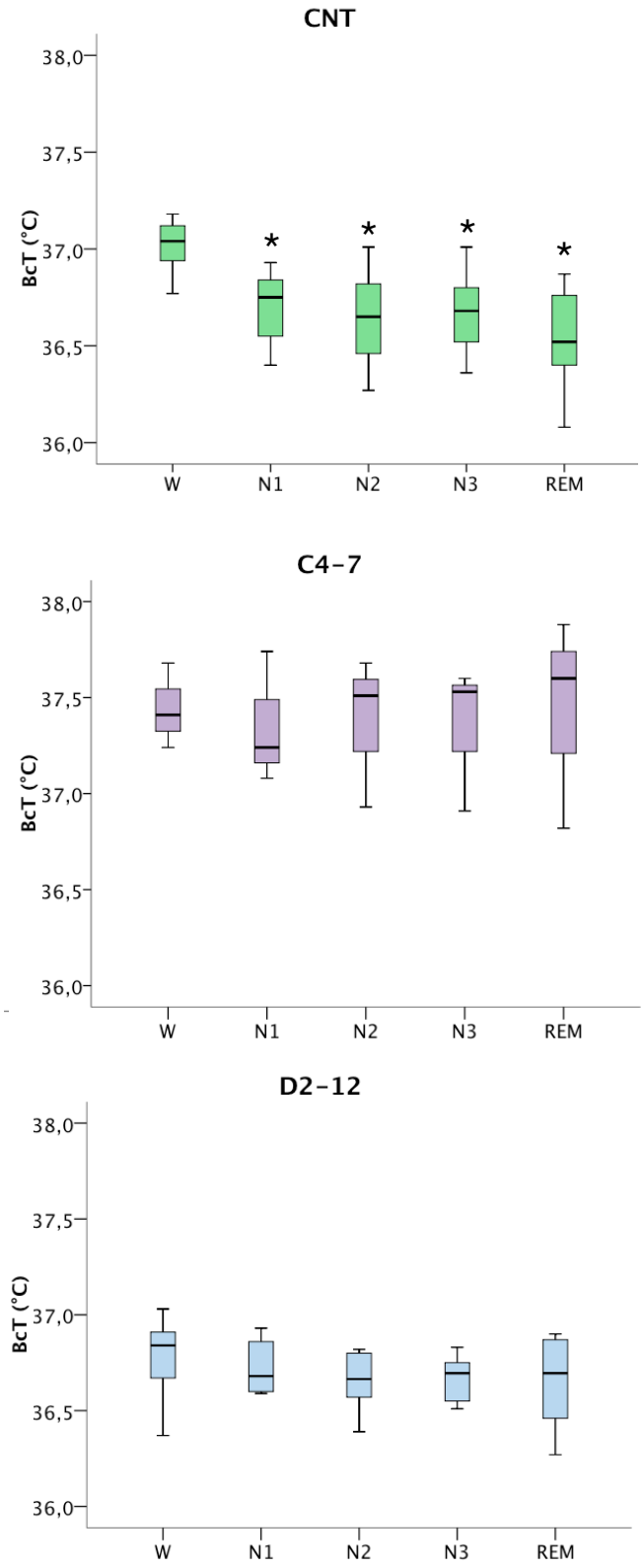


∗=p<0,01 vs cervical SCI.

BcT: body core temperature; C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; Δ: changes with respect to wake; N1: stage 1 NREM sleep; N2: stage 2 NREM sleep; N3: stage 3 NREM sleep; REM: REM sleep; SCI: spinal cord injury; W: wake.

APPENDIX: Section III

Fig III.7: state dependent changes in BcT in SCI patients and controls (within groups)



*=p<0,001 vs within group wake (W) values

BcT: body core Temperature; C4-7: cervical SCI; CNT: controls; D2-12: thoracic SCI; N1: stage 1 NREM sleep; N2: stage 2 NREM sleep; N3: stage 3 NREM sleep; REM: REM sleep; SCI: spinal cord injury; W: wake.

APPENDIX: Section III

ACKNOWLEDGEMENTS

Questa tesi di dottorato è il frutto del lavoro e della collaborazione di diverse persone. Ognuno nel suo singolo ha contribuito in maniera significativa e ha portato idee e contribuiti senza le quali questo risultato non sarebbe stato possibile.

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