



AARO SALMINEN

Peripheral Hypoxia
and Autonomic Responses
in Restless Legs Syndrome



ACADEMIC DISSERTATION

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University of Tampere, School of Medicine
Tampere University Hospital, Department of Respiratory Medicine
Unesta Research Centre
Finland

Supervised by
Docent Olli Polo
University of Tampere
Finland

Reviewed by
Docent Hannu Lauerma
University of Turku
Finland
Docent Tapani Keränen
University of Eastern Finland
Finland

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Abstract

Restless legs syndrome (RLS) is a circadian disorder with both sensory and motor components. It is characterized by an uncomfortable feeling in the legs that typically occurs in the evening time at rest, and urges movement. It is a hereditary disorder that affects up to 30% of Western populations at some point in their lives, and requires medical attention in 2% of the population. RLS may appear independently of other medical conditions, or related to pregnancy or renal failure. Most patients with RLS also have periodic leg movements (PLM) during sleep. Dopaminergic medication alleviates RLS and suppresses PLM efficiently.

The pathophysiology of RLS is currently not known. Most commonly, the origin has been suggested to be in the brain or spinal cord, but the findings have been slightly inconsistent. Therefore, other mechanisms have been suggested. Recent studies have demonstrated the activation of hypoxic pathways in the legs of RLS patients, suggesting peripheral involvement. However, the reason why these pathways are activated remains unknown. The aim of this work was to investigate potential hypoxia and hypercapnia in patients with RLS and the autonomic activations coupled to PLM.

The partial pressure of oxygen and carbon dioxide (ptO₂ and ptCO₂, respectively) was investigated in the skin tissues of the legs of 15 RLS patients and 14 healthy controls during provocation of RLS symptoms. Transcutaneous measurements were used to estimate the partial pressures. Simultaneously, skin temperature, oxy-hemoglobin saturation (SaO₂) and subjective discomfort were monitored. During the night, a polygraphic sleep study was performed to a subset of patients. The sleep study included recording of the plethysmogram signal from the toe in order to detect events of vasoconstriction.

The patients with RLS showed lower oxygen levels in their legs (5.54kPa vs. 7.19kPa, $p < 0.01$) but not on the chest (8.75kPa vs. 8.20kPa, $p = 0.355$), when compared to the control subjects. The oxygen level was found to correlate with the severity of RLS. The skin temperatures or the levels of SaO₂ or ptCO₂ did not differ between patients and controls. During sleep, the PLM were associated with transient events of vasoconstriction in a subset of RLS patients. The administration

of pramipexole raised the peripheral oxygen levels (from 5.54kPa to 6.65kPa, $p < 0.05$) and suppressed PLM and vasoconstriction events.

PLM were also investigated in a patient with a complete spinal cord injury with four polygraphic sleep recordings. The patient had a heavy burden of PLM at baseline. The PLM were temporally disconnected from the cortical and autonomic arousals. When a single dose of pramipexole was administered, the PLM were suppressed.

The data presented here provides evidence that hypoxia may be the reason behind the activation of hypoxic pathways in the periphery of RLS patients. The hypoxia could have a role in the pathogenesis of RLS, suggested by the connection with RLS severity. However, the hypoxia is not likely to be caused by reduced blood flow, as indicated by the normal skin temperature. Evidence suggests that pramipexole redirects blood flow to the periphery and resolves the local hypoxia. This could be a potential new site-of-action of dopaminergic therapy.

Regarding PLM, our data describes for the first time the transient peripheral vasoconstriction related to the leg movements. The effect of pramipexole implies that the vascular events are in tight causal relationship with the leg movements. On the other hand, it was shown that PLM may appear and may be suppressed by pramipexole without a connection to the brain. This suggests that the PLM in our patient are generated somewhere below the level of the lesion, either in the spinal cord or the periphery.

In conclusion, this work provides further evidence supporting the involvement of peripheral systems in the generation of both RLS symptoms and PLM. Possible systems involved in the peripheral aspects of RLS are the autonomic nervous system, oxygen transportation, blood circulation or hypoxic pathways. Although consistent, the findings should at this point be considered preliminary and need to be confirmed in future studies.

Tiivistelmä

Levottomat jalat -oireyhtymä (RLS) on yleinen sairaus, jolla on sekä sensorisia että motorisia ominaispiirteitä. Sen tyypillisimpiä oireita on jaloissa illalla esiintyvä epämiellyttävä tunne, joka pakottaa liikuttamaan jalkoja ja pahenee levossa. RLS on vahvasti perinnöllinen sairaus, josta kärsii elämänsä aikana jopa 30 % Länsimaiden asukkaista. Se kuitenkin vaatii hoitoa vain noin kahdella prosentilla väestöstä. RLS voi liittyä raskauteen tai munuaisten vajaatoimintaan, mutta se voi esiintyä myös ilman erillistä syytä. Suurimmalla osalla RLS:sta kärsivistä potilaista voidaan todeta periodisia jalkojenliikkeitä (PLM) unirekisteröinnissä. Dopamiinin tapaan vaikuttavat lääkkeet poistavat RLS-oireen sekä öiset jalkojenliikkeet tehokkaasti.

RLS-oireen alkuperää ei tiedetä. Yleisimmän hyväksytyn teorian mukaan oireen alkuperä on aivoissa tai selkäytimessä, mutta kaikki havainnot eivät ole tukeneet näitä väitteitä. Tämä on johtanut vaihtoehtoisten teorioiden kehitykseen. Viimeaikaiset tutkimustulokset ovat osoittaneet hypoksiaan liittyviä merkkejä RLS-potilaiden jalkojen lihaksissa, mikä voisi tarkoittaa hypoksian sekä periferisten järjestelmien keskeistä roolia RLS:ssa. Näiden löydösten mekanismeja ei kuitenkaan tunneta. Tämän työn tarkoitus oli tutkia mahdollista hypoksiaa ja hyperkapniaa sekä jalkojen liikkeisiin liittyviä autonomisia vasteita RLS-potilailla.

Koehenkilöiden jalkojen ja rinnan ihokudoksen happi- ja hiilidioksidiosapaineita mitattiin transkutaanisesti 15 RLS:sta kärsivällä potilaalla sekä 14 terveellä verrokkihenkilöllä oireiden provosoinnin yhteydessä. Samaan aikaan mitattiin ihon lämpötilaa, valtimoveren happisaturaatiota sekä itse raportoitua subjektiivista jalkojen epä mukavuutta. Yön aikana osalle koehenkilöistä tehtiin unirekisteröinti, johon kuului veren happisaturaation ja pletysmografian mittaaminen koehenkilön varpaasta. RLS-potilaat kävivät läpi mittaukset sekä ilman lääkitystä että pramipeksoli-lääkkeen ottamisen jälkeen.

RLS-potilaiden jalkojen happipitoisuus oli matalampi kuin kontrollihenkilöillä (5.54kPa vs. 7.19kPa, $p < 0.01$), mutta rinnalta mitattuna tilastollista eroa ei havaittu (8.75kPa vs. 8.20kPa, $p = 0.355$). Jalkojen happitasot olivat matalammat vakavammasta RLS-oireesta kärsivillä potilailla. Iholämpötiloissa tai hiilidioksidiosapaineissa ei ollut eroa ryhmien välillä. Unen aikana PLM-liikkeiden yhteydessä havaittiin hetkellisiä periferisiä vasokonstriktioita osalla potilaista.

Pramipeksoli nosti jalkojen happitasoa RLS-potilailla ja poisti unenaikaiset jalkojenliikkeet sekä niihin liittyvät vasokonstriktiot.

PLM-liikkeitä tutkittiin myös potilaalla, joka kärsi kroonisesta selkäydinvammasta. Potilaalle tehtiin kaksi unitutkimusta, joissa hänellä todettiin suuri määrä jalkojenliikkeitä huolimatta täydellisestä selkäytimen leesiosta. Liikkeet eivät olleet ajallisesti yhteydessä aivokuorella todettuihin havahduksiin tai sydämen sykkeen muutoksiin. Liikkeet saatiin poistettua matalalla annoksella pramipeksolia kolmannen unirekisteröinnin aikana.

Tässä työssä esitellyt tulokset tuovat uutta näyttöä siitä, että hypoksia on todennäköisin tekijä RLS-potilaiden jaloissa todettujen vaskulaaristen muutosten takana. Hypoksian suorasta yhteydestä oireen vakavuuteen voidaan päätellä, että se saattaa olla tärkeä tekijä RLS-oireen synnyssä. Hypoksiaa ei kuitenkaan näytä aiheuttavan vähentynyt periferinen verenkierto, kuten normaaleista iholämpötiloista voi päätellä. Rautametabolian muutokset tarjoavat vaihtoehdoisen selitysmallin näille löydöksille. Tuloksista voi myös päätellä, että dopamiini saattaa vaikuttaa veren virtaamiseen periferisiin kudoksiin, mikä saattaisi olla mahdollinen dopamiinin tapaan vaikuttavien lääkkeiden vaikutuskohta levottomat jalat - oireyhtymässä.

Tässä työssä kuvattiin myös ensimmäistä kertaa unenaikaisiin jalkojenliikkeisiin liittyvät periferiset vasokonstriktiot. Pramipeksolin vaikutus saattaa merkitä sitä, että havaitut vaskulaariset ilmiöt liittyvät suoraan jalkojenliikkeisiin. Pramipeksoli voi poistaa jalkojenliikkeet myös ilman yhteyttä aivoihin potilaalla, joka kärsii selkäydinvammasta. Tästä voi päätellä, että pramipeksolin vaikutuskohta oli tässä tapauksessa aivojen ulkopuolella, joko selkäytimessä tai periferisissä kudoksissa.

Yhteen vedettynä tässä työssä esitetään uutta todistusaineistoa periferisten kudosten roolista RLS:n sekä siihen liittyvien unenaikaisten jalkojenliikkeiden synnyssä. Tämä syntymekanismi saattaisi johtua niin autonomisesta hermostosta, hapenkuljetuksesta periferisiin kudoksiin, periferisestä verenkierrosta kuin hypoksiaan liittyvistä järjestelmistä. Vaikkakin tässä työssä esitetyt tulokset luovat johdonmukaisen kokonaisuuden, on niitä pidettävä alustavina löydöksinä ja niille on haettava vahvistusta uusista tutkimuksista.

List of original publications

- I. Salminen AV, Rimpilä V and Polo O. Peripheral hypoxia in restless legs syndrome (Willis-Ekbom disease). *Neurology* 2014; 82(21):1856-61.
- II. Salminen AV, Rimpilä V and Polo O. Pramipexole alters thermoregulation in restless legs syndrome. *Journal of Clinical Sleep Medicine* 2014; 10(12):1325-9.
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- IV. Salminen AV, Manconi M, Rimpilä V, Luoto TM, Koskinen E, Ferri R, Ohman J and Polo O. Disconnection between periodic leg movements and cortical arousals in spinal cord injury. *Journal of Clinical Sleep Medicine* 2013; 9(11):1207-9.

Abbreviations

AASM = American Academy of Sleep Medicine
ADHD = Attention-deficit/hyperactivity disorder
ANS = Autonomic nervous system
ASIA = American Spinal Injury Association
ATP = Adenosine triphosphate
AVA = Arterio-venous anastomose
BP = Blood pressure
BTBD9 = A gene associated with RLS
CAP = Cyclic alternating pattern
CGI-1 = Clinical global impression item 1
CNS = Central nervous system
COPD = Chronic obstructive pulmonary disease
CPAP = Continuous positive airway pressure
CVD = Cardiovascular disease
DPG = Distal-to-proximal skin temperature gradient
EEG = Electroencephalography
EKG = Electrocardiography
EMG = Electromyography
EOG = Electrooculography
ESS = Epworth sleepiness scale
HR = Heart rate
ICD = Impulse control disorder
ICSD-2 = International classification of sleep disorders
ISNCSCI = International Standards for Neurological Classification of Spinal Cord Injury
IRLSSG = International restless legs syndrome study group
IV = Intravenous
LBXCOR1 = A gene associated with RLS
L-DOPA = L-3,4-dihydroxyphenylalanine
MAP2K5 = A gene associated with RLS

MEIS1 = A gene associated with RLS
MRI = Magnetic resonance imaging
MS = Multiple sclerosis
nNOS = Neuronal nitric oxide synthase
NO = Nitric oxide
NREM = Non-rapid eye movement sleep
pCO₂ = Partial pressure of carbon dioxide
PD = Parkinson's disease
PLM = Periodic leg movements
PLMD = Periodic leg movement disorder
PLMI = Periodic leg movement index
PLMS = Periodic leg movements during sleep
PLMW = Periodic leg movements during wakefulness
pO₂ = Partial pressure of oxygen
PSG = Polysomnography
ptCO₂ = Tissue partial pressure of carbon dioxide
ptO₂ = Tissue partial pressure of oxygen
PWA = Pulse wave amplitude
REM = Rapid eye movement sleep
RLS = Restless legs syndrome
RLS-QoL = Restless legs syndrome quality of life questionnaire
SaO₂ = Arterial oxyhemoglobin saturation
SCI = Spinal cord injury
SIT = Suggested immobilization test
SSRI = Selective serotonin reuptake inhibitor
tcCO₂ = Transcutaneous carbon dioxide
tcO₂ = Transcutaneous oxygen
VEGF = Vascular endothelial growth factor
WASM = World Association of Sleep Medicine

Contents

1	Introduction	13
2	Review of the Literature	14
2.1	Restless Legs Syndrome.....	14
2.1.1	Diagnosis and severity.....	14
2.1.2	Epidemiology and genetics.....	16
2.1.3	Secondary forms of RLS.....	18
2.1.4	Pathophysiology	20
2.1.5	Treatment	22
2.1.6	Comorbidities	27
2.1.7	Peripheral aspects of RLS.....	29
2.2	Periodic Leg Movements.....	31
2.2.1	Recording and scoring.....	32
2.2.2	Description of movements.....	33
2.2.3	Epidemiology.....	34
2.2.4	Pathophysiology	36
2.3	Peripheral blood circulation.....	37
2.3.1	Cutaneous and muscular blood flow	37
2.3.2	Circadian variation	40
2.3.3	Measurement.....	41
3	Aims of the present study	47
4	Subjects and methods	48
4.1	Project 1: Peripheral hypoxia in RLS.....	48
4.1.1	Study subjects	48
4.1.2	Study design	48
4.1.3	Methods of analysis	52
4.1.4	Statistical methods	55
4.2	Project 2: PLM in Spinal Cord Injury.....	56
4.2.1	Case report	56
4.2.2	Measurements.....	57
4.2.3	Methods of analysis	57
5	Results	59
5.1	Project 1: Peripheral hypoxia in RLS.....	59

5.1.1	Demographics.....	59
5.1.2	Subjective discomfort during SIT	59
5.1.3	Peripheral partial pressure of oxygen during SIT	60
5.1.4	Peripheral partial pressure of carbon dioxide during SIT	62
5.1.5	Skin temperatures in RLS	64
5.1.6	Peripheral vasoconstriction related to PLM	65
5.2	Project 2: PLM in Spinal Cord Injury.....	68
6	Discussion.....	72
6.1	Methodology.....	72
6.1.1	Subjects.....	72
6.1.2	Study protocol	73
6.1.3	Transcutaneous measurements	74
6.1.4	Skin temperature measurements.....	75
6.2	Peripheral hypoxia and restless legs syndrome	76
6.3	The mechanism of periodic leg movements	80
7	Summary and conclusions.....	82

1 Introduction

Restless legs syndrome (RLS) is a disorder characterized by an uncomfortable feeling that is difficult to describe, typically occurring in the legs at night and relieved by movement. The diagnosis of RLS is based on an interview, and no specific objective markers are known, although most RLS patients show periodic leg movements (PLM) in sleep recordings. The origin of RLS symptoms and PLM has been commonly thought to be in the central nervous system, due to the quick and efficient suppression of the symptoms by dopaminergic therapy.

However, recent results have suggested that also peripheral systems may play a role in the generation of the RLS symptoms. First of all, the activation of hypoxic pathways in the skeletal muscle tissues of the legs of RLS patients could be a sign of peripheral hypoxia (Wåhlin-Larsson, Ulfberg et al. 2009). The same is suggested by the increased tortuosity of the vasculature in the same tissues (Wåhlin-Larsson, Kadi et al. 2007). There are many potential explanations for these findings. The most likely possibility is the presence of hypoxia, caused by changes in peripheral blood flow or metabolism. Blood flow to the periphery has been shown to be abnormal in RLS (Anderson, Di Maria et al. 2013), supporting this theory. However, these findings could be explained by other factors than hypoxia (Dery, Michaud et al. 2005). Therefore, the levels of oxygen and carbon dioxide should be measured directly in order to investigate the role of hypoxia in the activation of these pathways in RLS.

PLM, on the other hand, have been shown to be associated with transient autonomic activation, manifesting as elevations of heart rate (Ferri, Zucconi et al. 2007) and blood pressure (Pennestri, Montplaisir et al. 2007), as well as cyclic alternating pattern on cortical measurements (Parrino, Boselli et al. 1996). However, transient changes in peripheral sympathetic tone have never been measured in relation to PLM. This could increase our knowledge of the generation of the movements.

The general aim of this study was to investigate the peripheral phenomena related to RLS and PLM with the help of transcutaneous measurement of tissue gases, photoplethysmography and skin temperature. In addition, the properties of PLM were examined in complete spinal cord injury.

2 Review of the Literature

2.1 Restless Legs Syndrome

Restless legs syndrome (RLS) is a common sensorimotor disorder, characterized by uncomfortable feeling in limbs during rest and the initiation of sleep. (Trenkwalder, Paulus 2010) This may prolong the sleep onset or cause nocturnal awakenings. Consequently, the total sleep time in patients suffering from RLS is reduced (Hornyak, Feige et al. 2007), and it may lower the quality of life of the patients (Happe, Reese et al. 2009). RLS is a relatively common complaint, but requires medical intervention in only a fraction of sufferers.

The first known description of the symptoms of RLS was that of Thomas Willis, the physician of King Charles II of England, in his book “*De Anima Brutorum quae Hominis Vitalis ac Sentitiva est: Exercitationes Duae*” in 1672. However, the term 'restless legs' was not used before 1945 when it was coined by Karl-Axel Ekbom to describe eight of his patients (Ekbom 1945). At that moment, the word 'syndrome' was not yet used in the context of restless legs. In the honor of Dr. Ekbom, RLS is sometimes referred to as Ekbom disease. The syndrome has received more and more attention in the last decades due to improved quality of life and longer lifetimes.

Recently, the naming of RLS has been under heavy debate. Experts on RLS research argue that the understanding of RLS has now reached maturity, and that the symptoms and pathophysiology of RLS has been characterized well enough to call it a 'disease' instead of a 'syndrome' (Garcia-Borreguero 2012). Therefore, it has been suggested that RLS would be called Willis-Ekbom disease in the future, as homage to the two pioneers of the field. However, presently the term 'restless legs syndrome' is more used in the literature, and will also be used in this work.

2.1.1 Diagnosis and severity

The RLS research started to advance more rapidly after the agreement upon diagnostic criteria. Today, the diagnosis of RLS is based on criteria established by the International Restless Legs Syndrome Study Group (IRLSSG) in 2003 (Allen, Picchiatti et al. 2003). The essential diagnostic criteria of RLS include the following:

1. An urge to move the legs, usually accompanied or caused by uncomfortable and unpleasant sensations in the legs
2. The urge to move or unpleasant sensations begin or worsen during periods of rest or inactivity, such as lying or sitting
3. The urge to move or unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues
4. The urge to move or unpleasant sensations are worse in the evening or night than during the day or only occur in the evening or night (Allen, Picchiatti et al. 2003).

These diagnostic criteria are still in use. Recently, an additional essential criterion has been suggested, excluding common mimics of RLS, such as cramps and neuropathies (Hening, Allen et al. 2009). In particular, the symptoms should be differentiated from akathisia, a common condition induced by neuroleptics. In akathisia, however, the symptoms do not have a circadian component (Sachdev 1995). Therefore, a fifth criterion has been added to the essential diagnostic criteria:

5. The symptoms are not explained by other neurological conditions.

Additionally, RLS might be associated with the following additional clinical features, which may help in cases of diagnostic uncertainty (Allen, Picchiatti et al. 2003):

6. Positive family history of RLS
7. Response to treatment with dopaminergic drugs
8. Periodic limb movements in sleep.

The neurological examination in these patients is often normal, although peripheral neuropathy may be found in some patients (Ondo, Jankovic 1996). The symptoms often arise in middle age, and worsen with age, often causing increasing sleep disturbance (Allen, Picchiatti et al. 2003).

The severity of RLS can be evaluated by questionnaires, regarding the intensity of the subjective symptoms of RLS, such as the urge to move legs. IRLSSG has also developed a severity rating scale for clinical use (Walters, LeBrocq et al. 2003). The questionnaire ranks the severity of RLS based on ten questions. Each question is answered in the range of 0-4 representing increasing RLS severity, thus providing a final scale from 0 to 40. 1-10 points indicate mild, 11-20 moderate, 21-30 severe and 31-40 very severe RLS. The minimal clinically significant change on the rating scale for pharmaceutical studies is considered to be six points (Allen 2013). A factor analysis revealed two subscales within the IRLSSG (International Restless

Legs Syndrome Study Group) severity scale. (Allen, Kushida et al. 2003) One subscale can be used to measure symptom severity (6 questions) and the other to evaluate the symptom impact on daily living (4 questions).

Other questionnaires exist for evaluating RLS severity and impact. These include the RLS 6-item questionnaire (Kohnen, Oertel et al. 2003), and the RLS quality of life questionnaire (RLS-QoL) (Abetz, Vallow et al. 2005). A recent article compared these two questionnaires and a commonly used Clinical Global Impression item 1 (CGI-1) to the IRLSSG scale. The authors concluded that the inter-correlation of the questionnaires is good and that the IRLSSG scale together with CGI-1 is sufficient for evaluating RLS severity and impact in clinical trials (Allen, Oertel et al. 2013).

The suggested immobilization test (SIT) can be used as a specific tool for the diagnosis of RLS and the evaluation of its severity (Montplaisir, Boucher et al. 1998). In the SIT test, the patient sits on a bed while keeping the legs still for 60 minutes. During this time, possible voluntary or involuntary leg movements are detected through EMG recording of the anterior tibial muscle. The movements are then analyzed from the EMG recording with similar criteria that are used in evaluation of periodic leg movements (Michaud, Poirier et al. 2001). In patients suffering from RLS, periodic leg jerks may occur during the immobilization. A leg movement index can be computed to estimate the severity of RLS. A variation of this test, the forced immobilization test, may be used in some cases to assess the responses of the RLS patients to immobilization (Montplaisir, Boucher et al. 1998). However, this test may produce considerably more discomfort to the patient.

2.1.2 Epidemiology and genetics

The prevalence of RLS in Caucasian populations is evaluated between 4 and 29 % of the adult populations in different epidemiological studies (Innes, Selfe et al. 2011). In the USA, the largest study so far has estimated the prevalence as 5.8% in the general population (Gao, Schwarzschild et al. 2009). The prevalence of clinically significant RLS has been assessed in two large studies. In a European population the prevalence was reported at 2.1% (Allen, Stillman et al. 2010) whereas in North America the corresponding prevalence was 2.7% (Allen, Walters et al. 2005). The incidence of RLS has been estimated to be at 1.7% per year in a North American population (Budhiraja, Budhiraja et al. 2012) and at 2.2% in Germany (Szentkiralyi, Fendrich et al. 2011). Higher incidence of RLS has been associated with people in less favourable socio-economic conditions (Szentkiralyi,

Fendrich et al. 2012). In European studies, a prevalence of 8.5% was found in France (Tison, Crochard et al. 2005) and of 10.5% in Germany (Berger, Luedemann et al. 2004). In the only Finnish study, the prevalence was estimated to be 17.5% (Juuti, Laara et al. 2010) in a 57-year-old population in Northern Finland. In Southern Europe and in Asian countries, however, the prevalence of RLS is lower than in Northern Europe (Tan, Seah et al. 2001, Hadjigeorgiou, Stefanidis et al. 2007).

In addition to adults, RLS may also be found in children and adolescents. Slightly different diagnostic criteria are used for pediatric RLS than for adult RLS. Possible RLS may be diagnosed if the child is observed to seem restless or uncomfortable in situations precipitating RLS symptoms (Picchiatti, Bruni et al. 2013). The prevalence of definite or probable RLS in pediatric populations has been estimated to be up to 5.9% (Kotagal, Silber 2004). The significant overlap of symptoms with attention-deficit/hyperactivity disorder (ADHD), muscle pain and growing pains make the diagnosis challenging.

RLS is a highly hereditary condition, as established already by early familial aggregation studies. In these studies it was reported that a significant portion of patients suffering from idiopathic RLS also reported RLS among the first-degree relatives: the percentage of familial RLS among these patients has varied in different studies from 63% (Montplaisir, Boucher et al. 1997) to 92% (Ondo, Jankovic 1996). This is especially true for those patients with the onset of RLS at an early age, before 20 years old (Walters, Hickey et al. 1996).

In more recent genome-wide association studies, RLS has been associated with several genetic loci. A study in German and Canadian RLS populations showed associations with variants of MEIS1 and BTBD9 loci, as well as a locus including MAP2K5 and LBXCOR1 (Winkelmann, Schormair et al. 2007). Another study, focusing only on patients with RLS and objectively reported periodic leg movements, also found association with a common variant in the intron of BTBD9 (Stefansson, Rye et al. 2007). The results have been confirmed since in other populations, including the Finnish RLS patients (Kemlink, Polo et al. 2009, Yang, Li et al. 2011). In addition to these loci, also variants of the neuronal nitric oxide synthase (nNOS) gene have been associated with RLS in an association study (Winkelmann, Lichtner et al. 2008).

Recently, the MEIS1 locus, associated with RLS, has been further characterized from the neurodevelopmental viewpoint (Spieler, Kaffe et al. 2014). It was established in mouse and zebrafish models that the single-nucleotide polymorphism of MEIS1, previously associated with RLS, has a crucial role in the

development of the nervous system, in particular the forebrain. This indicates that the gene is active in the developmental phase of the telencephalon and not in the adult brain, suggesting that RLS could have elements of a neurodevelopmental disorder.

With the help of this information, there have been attempts to create an animal model of RLS in the mouse (Deandrade, Johnson et al. 2012) and the fruit fly (Freeman, Pranski et al. 2012), as well as in non-human primates (Barraud, Obeid et al. 2010). However, many challenges exist in the creation and validation of this model (Manconi, Hutchins et al. 2007). A major obstacle is the fact that RLS is defined as a set of subjectively reported symptoms, and no objective chemical or neurological markers of RLS exist that would be specific and accurate enough for testing. Until now, motor markers resembling PLM or nocturnal restlessness have been used in validation of these models (Manconi, Hutchins et al. 2007). When a valid animal model is created, it could be the perfect tool for studying disease aetiology and treatment options.

2.1.3 Secondary forms of RLS

RLS may exist as an idiopathic syndrome (with no association to any other concomitant disease) or secondary to some other condition or disease. The most common conditions increasing the incidence of RLS are end-stage renal disease, pregnancy and iron deficiency.

Patients with end-stage renal disease have been shown to have significantly increased prevalence of RLS, compared to the general population, estimated to be between 18.4% (Merlino, Piani et al. 2006) and 45.8% (Siddiqui, Kavanagh et al. 2005). RLS has been shown to decrease the individual's quality of life (Abetz, Allen et al. 2004), as well as to increase the mortality of RLS patients (Li, Wang et al. 2013). The same genetic variants, *MEIS1* and *BTBD9*, associated with idiopathic RLS, have been shown to be associated with uremic RLS patients (Schormair, Plag et al. 2011). In these patients, RLS is often resolved upon receiving a kidney transplant, fixing the initial renal problem (Azar, Hatefi et al. 2007).

RLS is a frequent but transient problem during pregnancy. The estimations of the prevalence of RLS among pregnant women range from 10.4% (Chen, Liou et al. 2012) to 34% (Uglane, Westad et al. 2011). The latest European studies show a prevalence of 29.6% (Sarberg, Josefsson et al. 2012). In most cases, the symptoms peak at the third trimester and are alleviated within days after delivery (Manconi, Govoni et al. 2004). Even if the symptoms disappear after delivery, these women

have a high risk to develop RLS later in life (Cesnik, Casetta et al. 2010). It has been suggested that the gender difference in RLS prevalence (Szentkiralyi, Fendrich et al. 2011) could be explained by pregnancy (Pantaleo, Hening et al. 2010). In this study, the researchers found no significant difference between the prevalence of RLS among women who never gave birth and men. It has been suggested that the connection between pregnancy and RLS could be due to an interaction between estradiol and dopamine (Pereira, Rocha e Silva et al. 2013). To date, no genetic studies exist in RLS secondary to pregnancy, but it was recently shown that family history of RLS is a predictor also for RLS during pregnancy (Hennessy, De La Torre 2013).

Another significant risk factor for RLS is iron deficiency anemia. The prevalence of RLS in patients with iron deficiency anemia has been reported to be up to nine times higher than in the general population (Allen, Auerbach et al. 2013). Treatment of the iron deficiency with IV or oral iron supplements often resolves RLS in these patients (Allen, Adler et al. 2011). Low ferritin levels and abnormal iron metabolism in the central nervous system have also been reported in patients with idiopathic RLS (Mizuno, Mihara et al. 2005), which makes the connection between iron homeostasis and RLS interesting.

In addition to these three most common types of secondary RLS, other disorders have been associated with an elevated prevalence of RLS. The most obvious of these conditions are perhaps different sleep disorders. RLS has been associated especially with obstructive sleep apnea (Lakshminarayanan, Paramasivan et al. 2005, Ohayon, Roth 2002) and narcolepsy (Plazzi, Ferri et al. 2012), but also with sleep-disordered breathing (Roux 2013).

RLS has also been associated with numerous neurological conditions. Most studies have been able to demonstrate the association between peripheral neuropathy and RLS (Luigetti, Del Grande et al. 2013, Cho, Na et al. 2013), with one study suggesting more prominent RLS only in the hereditary forms of neuropathy (Hattan, Chalk et al. 2009). RLS is also associated with Parkinson's disease (PD) (Ondo, Vuong et al. 2002), although the results are conflicting (Loo, Tan 2008). The relationship between RLS and PD is particularly interesting, as both disorders are effectively treated by dopamine agonists. The prevalence of RLS in the population of PD has been evaluated in several studies, the results ranging from 0% to 20.8%. Some studies suggest that the association could be iatrogenic, a result of long-term dopaminergic treatment rather than of PD itself (Lee, Shin et al. 2009, Calzetti, Angelini et al. 2013). In addition to Parkinsonism and neuropathy, also multiple sclerosis (MS) has been associated with RLS in several studies

(Schürks, Bussfeld 2013). The results obtained in patients with MS or PD may be biased by the difficult discrimination between the sensory symptoms of these conditions and RLS.

RLS may also be connected with different kinds of neuropathies, although this connection is still debated (Rajabally, Martey 2013). In epidemiological studies, RLS has been associated with peripheral axonal neuropathy (Iannaccone, Zucconi et al. 1995), painful polyneuropathy (Gemignani, Vitetta et al. 2013) and diabetic peripheral neuropathy (Cho, Na et al. 2013). Late-onset RLS with no family history has also been associated with loss of small sensory fibers in the periphery (Polydefkis, Allen et al. 2000).

Other medical conditions have been reported to increase the risk of RLS. Intestine and bowel diseases, such as irritable bowel syndrome (Borji, Fereshtehnejad et al. 2012), celiac disease (Moccia, Pellecchia et al. 2010) and Crohn's disease (Weinstock, Bosworth et al. 2010) have been connected with the increased prevalence of RLS in past studies. In addition, RLS is also suspected to be more prevalent among patients with type 2 diabetes (Merlino, Valente et al. 2010).

As another type of secondary RLS, the symptoms of RLS may also be induced by different kinds of medication (Hoque, Chesson 2010). The most common RLS-inducing medication is mirtazapine, which may provoke both symptoms of RLS (Markkula, Lauerma 1997) and PLM (Fulda, Kloiber et al. 2013) in healthy users. Also other antidepressants, especially the selective serotonin reuptake-inhibitors (SSRIs), have been shown to increase the risk of RLS (Rottach, Schaner et al. 2008). Other pharmaceutical therapies increasing the incidence of RLS include other histamine antagonists, dopamine antagonists and some antiepileptics (Hoque, Chesson 2010). However, akathisia is a more common side-effect of neuroleptics in clinical work, and may be confused with RLS symptoms (Sachdev 1995).

2.1.4 Pathophysiology

One of the early pioneers of RLS research, Karl-Axel Ekbom, suggested that RLS was of peripheral origin (Ekbom 1945). This was based on the amelioration of the symptoms with nitroglycerine. However, after it was discovered that dopaminergic medication provided a rapid suppression of RLS symptoms (Akpınar 1987), a central dopaminergic deficiency was suggested as the mechanism of RLS pathogenesis (Montplaisir, Godbout et al. 1986). This candidate for a

pathophysiological mechanism was borrowed from PD, another movement disorder effectively treated with dopaminergic therapy.

However, in the case of RLS, the site of action of the dopaminergic medication in the central nervous system (CNS) has not been identified. The studies looking for abnormalities in dopamine, serotonin and their metabolites in the cerebrospinal fluid of RLS patients have been negative (Earley, Hyland et al. 2001, Stiasny-Kolster, Moller et al. 2004). In addition, imaging studies have given inconsistent results (Ruottinen, Partinen et al. 2000), possibly suggesting a hyperdopaminergic state rather than a dopamine deficiency in the CNS. The same is suggested by studies of dopamine transporter protein in the striatum (Earley, Kuwabara et al. 2011) and dopamine receptors in the putamen (Connor, Wang et al. 2009). Despite the inconsistent evidence, the theory of dopaminergic abnormality in the CNS remains a widely recognized theory of the origin of RLS.

Because of the unsatisfactory evidence supporting a dopaminergic abnormality in the brain, alternative theories have been proposed. The next candidate for the location of the RLS pathology has been the spinal cord. According to the theory originally published by Clemens and co-workers (Clemens, Rye et al. 2006), RLS could be caused by the degeneration of descending spinal pathways from the A11 neurons in the hypothalamus. Cell group A11 is a dopaminergic nucleus projecting to the spinal cord and is the only source of spinal dopamine (Lindvall, Bjorklund et al. 1983). The authors suggest that these long projections to the spinal cord are susceptible to degradation through the normal process of ageing. The degradation process would result in dysfunction of spinal sensory and motor systems and produce RLS symptoms.

There are several studies supporting a spinal dysfunction in RLS. Abnormal spinal reflexes in RLS have been reported in multiple studies. The patients with RLS have increased spinal excitability (Bara-Jimenez, Aksu et al. 2000) and diminished inhibition of the spinal H-reflexes (Rijsman, Stam et al. 2005). Also group I nonreciprocal inhibition of spinal reflexes has been shown to be impaired in RLS (Scaglione, Vetrugno et al. 2008). On the other hand, a postmortem study found no signs of degradation in A11 neurons in the hypothalamus in autopsy studies of RLS patients (Earley, Allen et al. 2009), possibly undermining this theory. However, the descending pathways in the spinal cord have never been studied in detailed anatomical studies.

Also iron deficiency in the CNS has been suggested to be a factor in the pathogenesis of RLS (Allen, Earley 2007). This is supported by the findings from ultrasound (Godau, Wevers et al. 2008, Schmidauer, Sojer et al. 2005) and

neuroimaging studies (Allen, Barker et al. 2001, Earley, B Barker et al. 2006). These studies provide evidence for changes in iron metabolism of the basal ganglia of the brain in idiopathic RLS. Decreased ferritin levels in the brain of RLS patients also point to a central iron deficiency (Mizuno, Mihara et al. 2005). More recently, a study by Connor et al. reported differences in the cerebral profile of proteins managing iron transportation at the blood-brain barrier (Connor, Ponnuru et al. 2011). These results could indicate deficiencies in the transportation of iron to the brain in patients with RLS.

In addition to the direct evidence from imaging studies, a high incidence of RLS is reported in diseases and conditions that are known to be associated with iron pathologies. These conditions include for example iron-deficiency anemia (Allen, Auerbach et al. 2013) and pregnancy (Cesnik, Casetta et al. 2010). Iron deficiency has also been associated with increased responses to both acute and chronic pain in mouse models (Dowling, Klinker et al. 2009), possibly resembling human RLS.

The connection between iron metabolism and dopamine in RLS could be the major role of iron in the synthesis of dopamine. Iron is an important cofactor for the tyrosine hydroxylase enzyme, converting L-tyrosine into L-DOPA, a precursor of dopamine in the human body (Nelson, Cox 2005). Therefore, insufficient supply of iron could limit the production of dopamine in the brain. Despite the mounting evidence, deficiencies in iron metabolism in the central nervous system have not been able to explain all aspects of RLS.

In addition to the more popular theories on the pathophysiology of RLS, an inflammatory mechanism mediating the generation of the symptoms of RLS has also been suggested by a review article on the topic (Weinstock, Walters et al. 2012). It concluded that 95% of the conditions often associated with RLS are associated with inflammatory reactions. This could mean that the mechanism of RLS could involve inflammatory changes. However, no prospective studies have been done in support of this hypothesis.

2.1.5 Treatment

2.1.5.1 Dopaminergic treatment

The dopaminergic theory of RLS origin is supported by the efficient acute response when treating with levodopa, an endogenous precursor of dopamine (Trenkwalder, Stiasny et al. 1995, Beneš, Kurella et al. 1999). Levodopa is administered in combination with carbidopa in order to block its effects on the

peripheral metabolism. In long-term treatment, levodopa remains efficacious in 40% of patients with RLS, when administered as sustained-release levodopa/carbidopa (Trenkwalder, Collado Seidel et al. 2003).

Due to its rapid onset of action, levodopa is suitable for controlling occasional or sporadic RLS in situations where moving is limited, such as theatre or airplane. In daily use, however, it is no longer recommended due to its short duration of action predisposing to a phenomenon called augmentation. Augmentation means the reappearance of symptoms with either greater subjective intensity or earlier time of onset than prior to the dopaminergic treatment. The symptoms may also extend to other, previously asymptomatic body parts. It may occur within a few weeks of starting the therapy. The occurrence of augmentation in levodopa therapy has been confirmed since the first reports in a prospective study (Högl, Garcia-Borreguero et al. 2010) and in a community sample (Allen, Ondo et al. 2011). At high doses of 50/200 mg, levodopa/carbidopa has been reported to cause augmentation in up to 82% of the patients in long-term use (Allen, Earley 1996).

Dopamine agonists have currently replaced levodopa as the first-line treatment for RLS (Ferini-Strambi, Manconi 2009). The dopamine agonists have a longer time of efficacy than levodopa, and they relieve symptoms more efficiently for the duration of the whole night. The dopamine agonists can be divided into ergot and non-ergot derivatives. Only non-ergot-derived dopamine agonists are used today, because of the rare but severe adverse effects caused by ergot-derived medication (cabergoline and pergolide). The most severe adverse effects of these drugs include retroperitoneal, pericardial and pleuropulmonary fibrosis (Schade, Andersohn et al. 2007). Less commonly used examples of ergot derived dopamine agonists include lisuride and bromocriptine (Ferini-Strambi, Manconi 2009).

Pramipexole and ropinirole are the two most commonly used dopamine agonists in the treatment of RLS. The superiority against placebo treatment in the long and short term has been demonstrated in large-scale studies for both pramipexole (Oertel, Stiasny-Kolster et al. 2007, Winkelman, Sethi et al. 2006) and ropinirole (Trenkwalder, Garcia-Borreguero et al. 2004, Walters, Ondo et al. 2004). The usual initial doses for pramipexole and ropinirole are 0.125 mg and 0.25 mg, respectively (Ferini-Strambi, Manconi 2009).

Both pramipexole and ropinirole are preferential D3 dopamine receptor agonists. The D3 preferential agonists have been shown to be more efficacious in suppressing the symptoms of RLS than dopamine agonists with more affinity to the D2 receptor (Manconi, Ferri et al. 2011). The half-life of pramipexole is 8-12 hours in the human body. Ropinirole, on the other hand, has a shorter half-life of

5-6 hours. In terms of drug safety and tolerability in the treatment of RLS, it has been suggested that pramipexole should be favored over ropinirole (Quilici, Abrams et al. 2008). However, head-to-head studies comparing efficacy and safety have not been conducted and the data remains inconclusive.

More recently, a third non-ergot derived dopamine agonist, rotigotine, has been tested for RLS. Similarly to pramipexole, rotigotine is a D3 receptor preferential dopamine agonist (Scheller, Ullmer et al. 2009). However, unlike pramipexole or ropinirole, rotigotine is administered through the skin with a transdermal patch, which enables a more stable release of the agent throughout the day. The efficacy of rotigotine has been demonstrated in both short-term (Högl, Oertel et al. 2010, Hening, Allen et al. 2010) and long-term (Oertel, Trenkwalder et al. 2011) treatment.

Even if the dopamine agonists can relieve all the primary symptoms of RLS, they have failed to provide significant improvement in sleep parameters, (Ferri, Manconi et al. 2010). In addition to suppressing PLM, treatment with dopaminergic medication is not able to alleviate the abnormalities that are present in the microstructure of sleep of patients with RLS. The abnormalities are observed as high sleep instability and frequent discontinuation of sleep. These findings could suggest that dopamine agonists are not the optimal treatment for RLS.

Similarly to levodopa, also dopamine agonists are affected by the problem of augmentation. In a 6-month prospective study, the augmentation rate during pramipexole treatment was 9.2% (Högl, Garcia-Borreguero et al. 2011). In another study, the rate of definite augmentation cases was 24% for ropinirole and 11% for pramipexole in a community sample (Allen, Ondo et al. 2011). Although the rates are lower than those of levodopa, they are nevertheless remarkable. Transdermal rotigotine has a slightly lower risk of clinically relevant augmentation, demonstrated as 2.9% in a recent 6-month trial (Oertel, Trenkwalder et al. 2011, Beneš; Garcia-Borreguero et al. 2012).

Beside augmentation, the dopamine agonists have other known side effects. Odds ratio for adverse events has been on average 1.48 during pramipexole, 2.07 during ropinirole and 2.41 during rotigotine treatment (Hornyak, Trenkwalder et al. 2012). Most common adverse events leading to dropouts in clinical trials include fatigue, nausea, headache and somnolence (Högl, Garcia-Borreguero et al. 2011). With rotigotine patches, skin reactions on the application site are common (Oertel, Trenkwalder et al. 2011). Another severe side effect of dopamine agonists is impulse control disorder (ICD), including pathological gambling, compulsive shopping or eating and hypersexuality. ICD could occur in up to 32% of cases of

RLS patients treated with dopamine agonists (Cornelius, Tippmann-Peikert et al. 2010). Dopamine agonists are also associated with withdrawal syndrome when the treatment is stopped (Rabinak, Nirenberg 2010), although this has not been studied in the context of RLS.

2.1.5.2 Alpha-2-delta ligands

Due to the augmentation and the residual sleep problems related to dopaminergic treatment, alternative therapies for RLS are needed. It has been discovered that anticonvulsants binding as ligands to the $\alpha 2\delta$ subunit of the voltage-dependent calcium channels could be an efficient treatment for RLS. These drugs include gabapentin and pregabalin, which are most commonly used for epilepsy, pain control or anxiety disorders.

A superior efficacy of gabapentin against placebo for suppressing RLS symptoms has been demonstrated (Garcia-Borreguero, Larrosa et al. 2002). Gabapentin reduced the RLS symptoms on all rating scales, and was also associated with improvements in sleep parameters. Also the acute efficacy of gabapentin enacarbil, a prodrug of gabapentin, on RLS has already been demonstrated in large-scale trials (Lal, Ellenbogen et al. 2012, Lee, Ziman et al. 2011). Another $\alpha 2\delta$ ligand often used in the treatment of RLS is pregabalin (Garcia-Borreguero, Ferini-Strambi et al. 2012). Evidence for the efficacy of pregabalin in the treatment of RLS is provided by two different studies (Garcia-Borreguero, Larrosa et al. 2010, Allen, Chen et al. 2010). Both of them have shown improvement in RLS severity measures. The doses of pregabalin in these studies range from 150 to 450 mg/day.

A recent head-to-head study with one-year follow-up compared pregabalin (daily dose of 300 mg) to pramipexole at two different doses (0.25 and 0.5 mg) (Allen, Chen et al. 2014). In this large, one-year-long study, pregabalin was found to be more effective in the treatment of RLS symptoms than the lower dose of pramipexole or placebo. Most importantly, pregabalin was also found to cause significantly less augmentation than pramipexole or placebo, suggesting that the augmentation phenomenon is iatrogenic and possibly associated only with dopaminergic therapy.

2.1.5.3 Iron

For RLS patients with low ferritin levels, it is recommended to first try either oral or intravenous iron treatment. The efficacy of oral iron against placebo has

been demonstrated in RLS patients with iron deficiency (Wang, O'Reilly et al. 2009). Recently, a study by Lee et al. compared the efficacy of oral iron to pramipexole in 30 patients with RLS and low serum ferritin (15-50 ng/ml) (Lee, Lee et al. 2014). The authors found no difference in efficacy between the treatment options. The response rate, however, was not impressive. The authors concluded by suggesting combined treatment of dopamine agonists and iron supplement for the patients with iron deficiency and not responding to iron or dopamine treatment alone.

Intravenous iron has been also studied as a treatment option in the context of RLS with iron deficiency with moderately promising results (Grote, Leissner et al. 2009, Sloand, Shelly et al. 2004). These studies used either iron dextran or iron sucrose as the source of iron in the intravenous administration. A recent preliminary study also suggested good efficacy and safety in intravenous ferric carboxymaltose treatment in RLS (Allen, Adler et al. 2011). In this study, 46 patients with RLS were randomized to either IV iron or placebo. Further studies are under way to confirm the results. In addition to patients with idiopathic RLS, also patients with pregnancy-related RLS and low ferritin have been treated with intravenous iron supplement with good results (Vadasz, Ries et al. 2013).

Taken together, promising results have been obtained with both oral and intravenous iron treatment in patients with RLS and iron deficiency. However, larger studies are required to establish these as recommended treatment for RLS, especially in the case of idiopathic RLS.

2.1.5.4 Opioids

In cases of severe RLS that cannot be sufficiently controlled with dopaminergic medication, opioid treatment may be used for treatment. Opioids, such as morphine and codeine, are psychoactive drugs that are often used to treat acute pain. The evidence for the treatment of RLS with opioids is scarce. Recently, a 12-week randomized controlled trial showed the safety and efficacy of prolonged-release oxycodone-naloxone in 306 patients with severe RLS and failure of treatment with previous medication (Trenkwalder, Beneš et al. 2013). This was the first and only controlled study to study opioids in RLS in a larger patient sample.

In addition to this one randomized trial, some evidence exists for the efficacy of other opioids in RLS. Oxycodone has been studied in one randomized trial, suggesting significant reduction in both RLS and sleep complaints (Walters, Wagner et al. 1993). Treatment of RLS with methadone has been studied in refractory RLS (Ondo 2005) and in a longitudinal study (Silver, Allen et al. 2011),

providing promising results. The third opioid drug with evidence for the treatment of RLS is tramadol (Lauerma, Markkula 1999). This open-label study reported effective suppression of RLS symptoms with a daily tramadol dose of 50-150 mg. However, no randomized trial has been conducted to date on these opioids and therefore the evidence is insufficient for official treatment recommendations (Garcia-Borreguero, Ferini-Strambi et al. 2012).

2.1.5.5 Non-pharmacological treatment

Also non-pharmaceutical options have been studied for RLS treatment, although not as consistently as the pharmaceutical alternatives. In a 12-week study, an exercise program was found efficient to treat RLS, as compared to controls with no exercise (Aukerman, Aukerman et al. 2006). Also cognitive behavioral therapy has been shown favorable for both treated and non-treated RLS patients, in a study by Hornyak et al (Hornyak, Grossmann et al. 2008). In addition, good sleep hygiene might have a positive effect on RLS patients, and the patients should avoid alcohol and caffeine when possible. Anecdotal findings also recommend concentration, hot baths or other application of extreme temperatures to avoid the RLS symptoms (Mitchell 2011).

2.1.6 Comorbidities

Probably the most common comorbidity of RLS is the periodic leg movements (PLM) during sleep and wakefulness (Hornyak, Feige et al. 2007). The prevalence of PLM is estimated to be approximately 80% among patients with untreated RLS (Montplaisir, Boucher et al. 1997). The mean number of PLM per hour during sleep was 22.9 in a polysomnography study (Hornyak, Feige et al. 2007). Patients with more severe RLS have more cortical arousals related to the PLM compared to patients with less severe RLS (Hornyak, Feige et al. 2007). PLM may fragment sleep but are sometimes not considered to be a target for potential treatment by itself (Hornyak, Feige et al. 2006). It may be debated whether PLM are a comorbid condition or a finding related to RLS. Therefore, PLM will be discussed separately in more detail in Chapter 1.2.

RLS may also be associated with common conditions, such as cardiovascular disease (Walters, Rye 2009). In a recent study by Batool-Anwar et al. higher systolic and diastolic blood pressure was associated with RLS in middle-aged women (Batool-Anwar, Malhotra et al. 2011). In addition, the study demonstrated that patients with more frequent RLS symptoms had higher blood pressure levels than

those with less frequent symptoms. The data was controlled for other risk factors of hypertension, such as obesity, diabetes and age. The association between RLS and hypertension has been found also in other studies (Ferini-Strambi, Walters et al. 2013). The association, however, remains controversial, since not all studies have found an association between RLS and hypertension (Cosentino, Arico et al. 2012) or cardiovascular diseases (Winter, Schürks et al. 2012) exist.

The cardiovascular comorbidities could be linked to periodic leg movements (Nannapaneni, Ramar 2014), which are associated with intermittent nocturnal fluctuations in the diastolic and systolic blood pressure (Pennestri, Montplaisir et al. 2007). More pronounced responses in blood pressure are observed when the leg movements are associated with a cortical microarousal, as compared to movements not causing a major cortical response. Also intermittent elevations in heart rate are associated with periodic leg movements (Winkelman 1999), which could contribute to the cardiovascular risk.

Case reports also associate RLS or PLM with stroke (Lee, Kim et al. 2009, Sechi, Agnetti et al. 2008, Unrath, Kassubek 2006). In these cases, the PLM or RLS symptoms appeared directly after the patients suffered an ischemic stroke. However, these findings have not been confirmed in larger studies. The relationship may also exist in the other direction. Stroke might be more common in RLS patients than normal controls. This has been suggested by initial studies, but due to the small sample size the results did not reach statistical significance (Walters, Moussouttas et al. 2010). However, patients with RLS suffering a stroke have been shown to have worse clinical outcome than stroke patients with no RLS (Medeiros, de Bruin et al. 2011).

A German time sequence analysis study of cardiovascular risk factors and RLS found that the diagnosis of RLS was not a significant risk factor for later development of cardiovascular morbidities (Szentkiralyi, Volzke et al. 2013). On the other hand, cardiovascular disease did predict the later onset of RLS. This suggests that cardiovascular disease may be a risk factor for RLS and not vice versa.

RLS has been also associated with psychological comorbidities. Depression is a major comorbidity in RLS, demonstrated in several studies (Gupta, Lahan et al. 2013, Lee, Ramsey et al. 2014). Other studies have indicated that RLS is partly associated with depression but not all aspects of depressive disorder (Hornyak, Kopasz et al. 2005). A prospective cohort study in Germany showed the possible bidirectional relationship between the two conditions: depression was a risk factor for developing RLS and untreated RLS was a risk factor for depression

(Szentkiralyi, Volzke et al. 2013). In addition to depression, RLS is also associated with elevated anxiety both in adult (Scholz, Beneš et al. 2011, Winkelman, Finn et al. 2006) and adolescent patient populations (Pullen, Wall et al. 2011). However, RLS does not affect cognitive functioning (Lee, Ramsey et al. 2014).

Other significant neurological comorbidities in RLS include migraine and headaches (Rhode, Hosing et al. 2007, Gupta, Lahan et al. 2012). In epidemiologic studies, women and men with migraine have been reported to have a minor but significantly increased risk to also suffer from RLS (Schürks, Winter et al. 2012, Winter, Schürks et al. 2013). Other studies report no increased risk but increased severity of RLS in patients with migraine (Gozubatik-Celik, Benbir et al. 2014). Other conditions linked with RLS include erectile dysfunction (Li, Batool-Anwar et al. 2013), sleep bruxism (Lavigne, Montplaisir 1994) and irritable bowel syndrome (Weinstock, Walters 2011).

2.1.7 Peripheral aspects of RLS

In contrast to the current pathophysiological models of RLS, several findings have recently suggested peripheral abnormalities in patients with RLS. The most prominent ones of these studies have been performed by Wåhlin-Larsson et al. In their first study, they showed the high capillary tortuosity in the skeletal muscles of the legs of patients with RLS (Wåhlin-Larsson, Kadi et al. 2007). In this study, the morphology of the skeletal muscles was assessed in 20 patients and 16 controls from muscle biopsies. The patients with RLS had significantly higher predicted maximal oxygen intake and tortuosity index, compared to the controls. In a subsequent study, the same group studied the levels of vascular endothelial growth factor (VEGF) in the same skeletal muscles of 12 patients with RLS (Wåhlin-Larsson, Ulfberg et al. 2009). The results showed higher expression of VEGF in the muscles of RLS patients, as compared to the healthy controls. Also the percentage of proliferating capillaries was higher in RLS. In addition, similar findings have been reported in the substantia nigra of patients with RLS (Patton, Ponnuru et al. 2011). Although not done in a peripheral tissue, this study provides additional evidence to the link between signs of hypoxia and RLS. Even if these markers are considered to be signs of hypoxia in the affected tissues, the hypoxic pathways may also be activated in normoxic conditions (Dery, Michaud et al. 2005).

Also the limb blood flow has been measured in RLS patients in two different studies. A study by Anderson et al. investigating the peripheral phenomena in RLS

found microvascular changes in the legs of RLS patients in skin temperature and laser-Doppler flowmetry measurements (Anderson, Di Maria et al. 2013). These findings could suggest impaired peripheral blood flow in RLS, if confirmed in other studies. The major limitation of the study is the fact that the RLS patients studied were on dopaminergic medication during the measurements. Another study by Oskarsson et al. used laser-Doppler flowmetry to examine intramuscular blood flow in RLS (Oskarsson, Wahlin-Larsson et al. 2014). They did not find a difference between RLS patients and controls, but found that the patients had higher blood flow to the extremities in the morning than in the evening. Also this study enrolled RLS patients with dopaminergic medication, without a sufficiently long wash-out period.

Changes in iron metabolism could serve as a potential explanation to these findings. If the iron metabolism is compromised not only in the basal ganglia of the brain but also in the periphery, it could result in the impairment of the delivery of oxygen to the peripheral tissues. To date, only one study has prospectively studied the iron in the systemic blood circulation in RLS. It demonstrated that the iron metabolism in blood lymphocytes is, indeed, impaired (Earley, Ponnuru et al. 2008). Measurements of hemoglobin have also been done in patients with RLS. While the hemoglobin levels were in the normal range in patients with idiopathic RLS (Arunthari, Kaplan et al. 2010), low hemoglobin was found to be a predictor of RLS secondary to pregnancy (Tunc, Karadag et al. 2007).

Another set of data, possibly supporting the existence of peripheral pathology in RLS, is provided by the usage of peripheral sequential compression devices in the treatment of RLS. Two studies have been done with such devices, both providing preliminary results of the beneficial effect in reducing RLS symptoms (Lettieri, Eliasson 2009, Eliasson, Lettieri 2007). In addition to compression devices, also enhanced external counter pulsation (EECP) techniques have been tested in RLS. Initial results with this technique were positive (Rajaram, Shanahan et al. 2005), but subsequent study by the same research group, unfortunately stopped prematurely, failed to find more support to the preliminary results (Rajaram, Rudzinskiy et al. 2006).

Several other studies add evidence to the involvement of hypoxic systems in RLS. The high prevalence of RLS in chronic obstructive pulmonary disease (COPD), especially in the highly hypoxic cases (Kaplan, Inonu et al. 2008), could suggest a role of hypoxia in RLS. Also patients suffering from chronic venous disorders have a high prevalence of RLS (McDonagh, King et al. 2007). This supports the hypothesis that peripheral blood perfusion plays a role in RLS. Some

evidence exists also for the association of RLS in patients living at high altitudes (Finsterer 2007, Sevim, Dogu et al. 2003), supporting the role of hypoxia in RLS. In addition, knee prostheses have been reported to cause subsequent RLS, supporting a peripheral mechanism (Pereira, Silva Neto et al. 2011). Genetic studies have shown the possible involvement of neuronal nitric oxide synthase (nNOS) in RLS (Winkelmann, Lichtner et al. 2008). The nNOS enzyme is involved in vasodilatation in the periphery, and could thus support a peripheral abnormality in RLS.

Taken together, previous literature provides indirect evidence for the presence of peripheral hypoxia in patients with RLS. However, as other explanations exist for the activation of hypoxic pathways, direct evidence of hypoxia is needed. This is important also in order to determine the role of the possible hypoxia in RLS: is it a primary trigger of RLS symptoms or a secondary phenomenon? Despite this dilemma, the oxygen content or oxygen delivery has not been measured in these tissues. This information could be valuable for our understanding of RLS, regardless of the role of oxygen delivery in the pathophysiology.

2.2 Periodic Leg Movements

PLM are a common feature in RLS, observed during sleep in most RLS patients (Montplaisir, Boucher et al. 1997). PLM are involuntary jerk-like limb movements or muscle contractions occurring both during sleep and wakefulness (Figure 1). PLM during sleep (PLMS) may cause arousals from sleep (Lavoie, de Bilbao et al. 2004), but may also occur without visible arousals in the EEG. The number and intensity of the movements vary from night to night, but diagnosis is possible based on one sleep recording, according to a study conducted with a relatively small sample size (Sforza, Haba-Rubio 2005). Also overnight variability occurs in PLM. Two distinct patterns have been differentiated in the distribution of PLM during the night (Culpepper, Badia et al. 1992). One group of patients has PLM primarily during the first part of the night, with fewer movements towards morning hours. In the other group, PLMs are evenly distributed through the duration of the night. In both groups, however, PLM were observed almost exclusively during non-REM sleep.

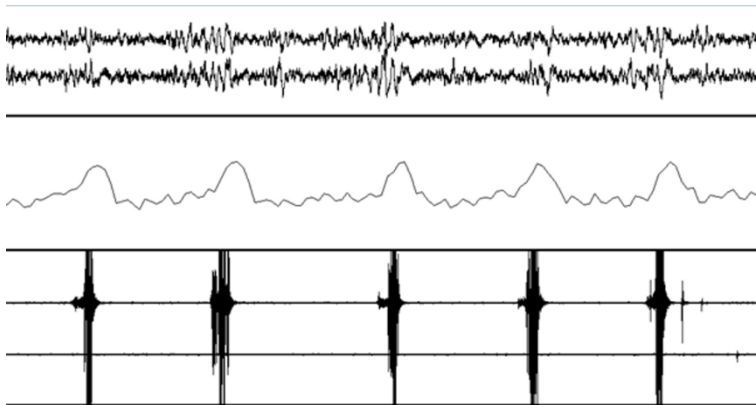


Figure 1. Typical PLM are shown in an extract from a sleep recording of an RLS patient. The example demonstrates the heart rate responses and cortical arousals associated with PLM. The channels shown are, from top to bottom, EEG C3-Fz, EEG C4-Fz, heart rate, EMG tibialis anterior right and EMG tibialis anterior left.

PLM may also appear during wakefulness. PLM during wakefulness (PLMW) appears often in patients with RLS (Hornyak, Feige et al. 2007), but may be present in recordings of other subject groups. The time structure of PLMW differs from that during sleep (Ferri, Manconi et al. 2012): the intervals between movements are generally shorter when the patient is awake. The appearance of PLMW predicts the PLMS in patients with RLS (Ferri, Manconi et al. 2012). In fact, it has been suggested that PLMW and PLMS share the same mechanism, based on the observation that the PLM often continue after sleep onset without a break (Earley, Allen et al. 2000).

2.2.1 Recording and scoring

PLM are conventionally detected in a polygraphic sleep recording, with the help of EMG electrodes attached on the skin, on the anterior tibial muscle on both legs (The Atlas Task Force 1993). Alternatively, the movements may be detected with actigraphy (Kazenwadel, Pollmächer et al. 1995). The actigraph devices should be attached to the toe in order to detect all movement (Kemlink, Pretl et al. 2008). Validation studies of actigraphs for the detection of PLM have resulted in different results. Some studies have found actigraphy to be a suitable method for the detection of PLM (Kemlink, Pretl et al. 2008), whereas others did not recommend the usage in longitudinal studies (Gschliesser, Frauscher et al. 2009).

The leg movements are analyzed from these recordings according to the criteria suggested by the World Association of Sleep Medicine (Zucconi, Ferri et al. 2006).

A leg movement is scored if the duration of the movement is between 0.5-10 seconds and the maximum amplitude is at least 8 mV above resting EMG. Periodic leg movement series are marked when at least 4 movements occur with inter-movement intervals of 5-90 seconds. Movements temporally associated with respiratory events (termination of episodes of apnea or hypopnea) are not scored. A cortical arousal is associated to the leg movement if it occurs within 0.5 seconds before or after the movement.

2.2.2 Description of movements

One of the principal properties of PLM is their periodicity. The periodicity has recently been extensively studied with a novel method (Ferri, Zucconi et al. 2006). In patients with RLS, PLMS show a distinct time pattern: most of the time, the movements repeat with steady 20-30-second intervals (Ferri, Zucconi et al. 2006). The distribution shows another peak at 0-10 seconds, possibly associated with sleep disruption as it can be observed also during wakefulness (Ferri, Manconi et al. 2012). This peak at shorter intervals may also be seen in healthy control patients, unlike the RLS-related peak at 20-30 seconds (Ferri, Zucconi et al. 2006). With this new method, a periodicity index may be computed (Ferri, Zucconi et al. 2006). This index has been shown to be more stable in repeated sleep recordings than the commonly used PLM index (Ferri, Fulda et al. 2013).

PLM are not appearing independent of other phenomena observed in polygraphic sleep recordings. They are often temporally coupled with arousals (Ferri, Zucconi et al. 2007) or cyclic alternating pattern (CAP) (Parrino, Boselli et al. 1996) observed in simultaneous EEG recording. EEG arousals disrupt sleep or cause awakenings. PLM-induced sleep disturbance could be a reason for therapeutic intervention if the patient complains of daytime sleepiness. CAP, on the other hand, is associated with the activation of sympathetic nervous system (Ferini-Strambi, Bianchi et al. 2000) and may be found also in healthy individuals.

The sympathetic intrusion coupled with PLM is also demonstrated by elevations in heart rate (Ferri, Zucconi et al. 2007) at the time of the movements. During NREM sleep, PLM may be associated with heart rate elevations of up to 20% from the baseline. In addition to heart rate, also blood pressure shows transient elevations related to PLM (Pennestri, Montplaisir et al. 2007). The mean elevation in systolic blood pressure was reported as 25 mmHg, while the rise in diastolic blood pressure was 10 mmHg. These elevations may even herald the leg movement by several seconds (Ferrillo, Beelke et al. 2004), suggesting that they are

not triggered by the leg movements. In addition to heart rate and blood pressure elevations, PLM have also been associated with vasoconstriction in an anecdotal report (Ware, Blumoff et al. 1988). However, this has not been confirmed in systematic studies.

Similarly to RLS, PLM observed in the sleep recordings of patients with RLS are effectively suppressed by dopaminergic treatment (Montplaisir, Godbout et al. 1986). The effect is visible already after the first dose of pramipexole, taken at least two hours before the bedtime (Manconi, Ferri et al. 2007). In this trial, the PLM index was shown to drop from 50.4/h to 10.4/h with acute pramipexole treatment. No data exists on the effect of long-term pramipexole therapy on PLM. On the other hand, pregabalin has a considerably smaller effect on PLM, suppressing some but not all of them in polysomnogram studies (Garcia-Borreguero, Larrosa et al. 2010). There is currently no data from larger trials on the effect of opioids on PLM. However, a smaller study reported a reduction of PLM after treatment with various opioids (Kavey, Walters et al. 1988).

Even if dopaminergic medication efficiently suppresses PLM, they have no effect on the EEG arousals or CAP temporally associated with the leg movements (Manconi, Ferri et al. 2012). On the other hand, when given clonazepam, a hypnotic drug of the benzodiazepine class, the arousals were suppressed while the PLM were not affected. This could mean that both of these forms of therapy are treating the observed abnormality only partially.

Since dopaminergic drugs efficiently suppress PLM, they remain an option, should suppression of PLM come into consideration. However, there is no consensus on whether the patient would benefit from treatment of PLM alone, in the absence of RLS symptoms.

2.2.3 Epidemiology

The prevalence of PLM in the general population has been estimated to be between 4-11% (Hornyak, Feige et al. 2006). However, no large scale polysomnographic study has been performed so far. A telephone survey estimated the prevalence of PLM, accompanied by sleep complaints, at 3.9% in the general population (Ohayon, Roth 2002). The appearance of PLMS tends to increase with age (Hornyak, Trenkwalder 2004, Bliwise, Carskadon et al. 1988) and it has been estimated that up to 80% of elderly patients with sleep difficulties have a PLM index above 5/h (Youngstedt, Kripke et al. 1998).

The most common association of PLM is the one with RLS, as more than 80% of RLS patients have been shown to also have PLM (Montplaisir, Boucher et al. 1997). The remaining 20% could be partially explained by night-to-night variation (Sforza, Haba-Rubio 2005). In addition to RLS, PLMS are a common finding in patients with other sleep disorders, such as narcolepsy (Baker, Guilleminault et al. 1986), obstructive sleep apnea (Hornyak, Feige et al. 2006) and REM sleep behavior disorder (Fantini, Michaud et al. 2002). In addition, the suppression of sleep apnea with continuous positive airway pressure (CPAP) treatment has been shown to increase the appearance of PLMS (Seo, Guilleminault 2012). Without RLS, PLM are also commonly found in disorders such as essential hypertension (Espinosa-Sierra, Vela-Bueno et al. 1997) and end-stage renal disease (Benz, Pressman et al. 2000), and may correlate with the worsening of these conditions.

In a more surprising note, PLM during sleep are also common among patients with complete or partial spinal cord injury (SCI). This has been reported previously in case reports (Yokota, Hirose et al. 1991) and later in larger samples (Telles, Alves et al. 2011). A mean PLMI of about 18/h was found in 24 patients with complete SCI. Up to 75% of the patients studied had PLMI above 5/h. These were significantly higher numbers than what are observed in the general population.

PLM may also be a side effect of medication. In a recent study, Fulda et al were able to demonstrate the appearance of PLM in sleep recordings of young and healthy men after short term exposure to mirtazapine, an antidepressant drug with antihistaminergic properties (Fulda, Kloiber et al. 2013). The PLM index was especially high on the first night after starting mirtazapine. The connection between PLM and mirtazapine has been reported previously in a case report (Mattoo, Mahajan et al. 2013).

The impact of PLM on sleep quality or daytime somnolence has been under debate (Hornyak, Feige et al. 2006). Several studies have reported no connection between PLMS and impairment of sleep, measured by subjective sleep complaints, daytime sleep latency tests or objective sleep measures in PSG (Hilbert, Mohsenin 2003, Mendelson 1996). However, two studies report a weak association between PLMI and subjective sleep quality (Hornyak, Feige et al. 2007, Carrier, Frenette et al. 2005). Therefore, treatment of PLM without subjective RLS complaints with dopaminergic drugs is not common practice.

The occurrence of PLM is associated with an increased risk of cardiovascular diseases (CVD), such as cerebral vascular disease or coronary heart disease (Koo, Blackwell et al. 2011). RLS patients with frequent PLM have also been shown to be

at higher risk of with left ventricular hypertrophy than those with less PLM (Mirza, Shen et al. 2013). In addition, PLM have been connected with atrial fibrillation (Mirza, Shen et al. 2013) and cardiac arrhythmia (Koo, Mehra et al. 2014) in certain populations. However, the mechanism of these associations is not known. It has been suggested that the elevated risk for CVD could result from the transient increases in heart rate (Ferri, Zucconi et al. 2007) and blood pressure (Pennestri, Montplaisir et al. 2007), associated with PLM. More prospective studies are warranted to further explore this association.

A heavy burden of PLM, observed in the sleep recordings in the absence of RLS complaints, is often labeled as periodic leg movement disorder (PLMD) and may provide an explanation to previously unexplained insomnia (Ferri, Gschliesser et al. 2009). According to the commonly used diagnostic criteria, PLMD may be diagnosed if PLMI exceeds 15/h and there is a clinical sleep disturbance that is not explained by RLS or other medical disorder (Hornyak, Feige et al. 2006). There has been no consensus within the scientific community, whether PLMD should be classified as a sleep disorder, and whether it should be treated in adults. On the other hand, the disorder has been well established and shown to have important clinical correlates in pediatric populations (Gingras, Gaultney et al. 2011).

2.2.4 Pathophysiology

The origin of PLM is unknown. However, several mechanisms have been suggested by different authors. It has been suggested that RLS and PLM share in part a common mechanism, as the movements often continue without a pause upon transition from wakefulness to sleep (Earley, Allen et al. 2000). As is the case with RLS, the pathology of PLM has been suggested to be in the central dopaminergic systems, due to the quick suppression of PLM by dopaminergic medication (Montplaisir, Godbout et al. 1986). This theory has been the prevailing theory for long, until recently, as challenging theories have been proposed.

One of the new theories suggests a spinal pacemaker as the trigger mechanism of PLM (Manconi, Ferri et al. 2012). Due to the pharmacological dissociation between cortical arousals and PLM, the authors have suggested that there are two distinct biological pacemakers: one responsible for the periodicity of the PLM and the other of the CAP and cortical arousals. These two pacemakers would be synchronized with one another through complex neural networks. The authors hypothesize that the pacemakers would be located in the sympathetic branch of the autonomic nervous system (Manconi, Ferri et al. 2012).

PLM could also be spinal reflexes to a peripheral stimulus, as suggested by Yokota et al. (Yokota, Hirose et al. 1991). This is supported by the fact that the pattern of muscle activation in PLM is similar to that of spinal flexor reflexes (Bara-Jimenez, Aksu et al. 2000). The same study evaluated the excitability of the flexor reflex in RLS patients who also had PLM. The reflexes were found to be significantly pronounced in the patients, and the difference with healthy controls was particularly prominent during sleep. This could indicate increased excitability of the spinal cord in these patients, especially during sleep. This finding is in line with the thinking that PLM are spinal reflexes to either normal or abnormal stimulus from the periphery.

Despite the attempts, no consensus has been found in the scientific community about the origin of the PLM. Therefore, further studies are needed before the mechanism responsible for the generation of PLM will be unveiled.

2.3 Peripheral blood circulation

The basic principle of the blood circulation in humans is relatively simple. The heart has the function of a pump, propelling blood into the major arteries. The blood flows to the tissues through smaller arteries that become smaller and smaller in diameter before reaching the capillary bed of the target tissues. When reaching the tissue, nutrients, oxygen and heat carried by the blood diffuse into the tissue. The oxygen-deprived blood then flows back towards the heart through veins (Boron, Boulpaep 2005).

The blood circulation in humans is organized into three different systems. The pulmonary circulation directs low-oxygen-content blood into the lungs in order to re-saturate it with oxygen. The coronary circulation supplies oxygen and nutrients to the heart muscle. By far the largest circulation system, the systemic circulation, is responsible for providing all other tissues with oxygen and nutrients. This chapter is focused on the most peripheral parts of the systemic circulation, primarily the blood circulation of skin and skeletal muscle tissues.

2.3.1 Cutaneous and muscular blood flow

The cutaneous microcirculation has three principal functions in the human body (Rossi, Carpi et al. 2006). The first function is, evidently, the transportation of nutrition and oxygen to the skin tissue and removing carbon dioxide. The second element is heat exchange. This is important for the thermoregulation of the body,

as the human body dissipates excess heat to the surroundings through the skin. The third function of the skin circulation is the redistribution of blood flow during “fight-or-flight” events or stress. This is important in order to direct blood flow to the tissues in need or energy. These functions are regulated by a fine control system adapting the blood flow to the skin through vasoconstriction and vasodilation. Therefore, the cutaneous vascular bed may be roughly divided into two kinds of blood vessel systems: the nutritional and the thermoregulatory vascular circuits.

The principal sites of regulation in the skin vascular circuit are the arterioles leading to the target tissue and the arterio-venous anastomoses (AVAs). These systems of blood flow regulation are located in series, as opposed to being parallel control mechanisms. The AVAs are densely innervated blood vessels bypassing the capillary system (Morris 1997, Morris 1997). They are the primary sites of cutaneous blood flow regulation: when the AVAs are dilated, most of the blood flowing to the skin bypasses the capillaries and flows directly to the veins. If AVAs constrict but the blood flow to the vascular bed remains stable, blood is forced to the capillaries, transporting more nutrients to the outer layers of the skin tissue.

Reflexes to thermal stimuli are good examples of thermoregulatory responses in the human skin. Cooling of the skin produces a constriction response in both AVAs and arteries leading to the skin tissue (Hales, Fawcett et al. 1978). The response is mediated by sympathetic neurons (Hales, Foldes et al. 1982). This way, the blood flow is redirected away from the skin affected by the cooling in order to minimize heat loss. Upon heating the skin, on the other hand, skin blood flow is generally increased by an opposite reaction. This reaction is achieved either through reduction of the sympathetic vasoconstrictive activity or through locally regulated parasympathetic vasodilation (Saumet, Degoute et al. 1992).

The main regulatory neurotransmitters, promoting sympathetic vasoconstriction in the cutaneous vascular bed, are noradrenaline, neuropeptide Y and adenosine triphosphate (ATP). Parasympathetic vasodilation, on the other hand, is predominantly conveyed by acetylcholine. Both adrenoceptors, reacting to noradrenaline, and purine receptors, reacting to ATP, are found in most arteries and mediate vasoconstriction. The receptors of neuropeptide Y, on the other hand, do not seem to have an influence on cutaneous AVAs. In other arteries, they produce a vasoconstriction of slightly lower maximal intensity than noradrenaline. Other neurotransmitters affecting the skin blood flow through vasoconstriction are serotonin and endothelin-1 (Morris 1997).

The regulatory mechanisms of blood flow to the skeletal muscles are slightly different than those in the skin tissue. During rest, the skeletal muscles receive about 15-20% of the total cardiac output. During heavy exercise, however, this figure may rise to 80-90% and the blood flow in a single contracting skeletal muscle may increase 50-fold (Buckwalter, Clifford 2001). Because of the high variation of blood flow between different states, more adaptability is required also from the regulatory systems. Therefore, the blood flow to the muscle is regulated by efferent sympathetic vasoconstrictive nerves as well as by local regulatory means, including rapid production and release of vasodilatory substances such as nitric oxide (NO). Even if sympathetic influence does have an effect on the muscle blood flow, the major adaptations of the vascular tone are achieved through locally induced vasodilation (Clifford, Hellsten 2004).

A good example of such transition is the beginning of exercise. During long-lasting dynamic exercise, skeletal muscles are doing active work whereas the skin is metabolically less active. For this reason, more blood flow is needed in the muscle than in the skin. The shift of blood between tissues is achieved by vasoconstriction of the skin vessels and local vasodilation of the vasculature of the muscle (Johnson 1986, Clifford, Hellsten 2004). However, the cutaneous vasoconstriction is only present during high-intensity dynamic exercise, and not during isometric exercise, low-intensity dynamic exercise or exercise involving only small muscle groups (Taylor, Johnson et al. 1990). There is evidence of a critical threshold of oxygen consumption above which the skin vasculature is triggered to constrict (Smolander, Saalo et al. 1991). To direct the blood flow more specifically to the contracting skeletal muscles, the arteries in and leading to those muscles are dilated. This dilation is mediated by local mechanism rather than neurogenic central devices (Clifford 2011). When exercise continues, distal skin vessels are also dilated in order to enhance heat loss through the extremities (Kellogg, Johnson et al. 1993).

Blood flow may be modified pharmacologically with vasodilator and vasoconstrictor substances. The most interesting of these, in the context of RLS, is dopamine. Dopamine, in addition to its effects in the central nervous system, is also involved in the regulation of blood flow. Dopamine receptors are present in abundance outside the central nervous system, with strong presence in systemic arteries (Amenta, Barili et al. 2000). Eliassen et al. showed in 1989 that dopamine could enhance the blood flow of the skeletal muscle and skin tissues of humans when administered intravenously (Eliassen, Klemp et al. 1989). The authors concluded that dopamine redirects blood flow from central parts of the body towards the periphery. Conflicting results have been obtained in animal studies,

where dopamine seemed to reduce blood flow in resting peripheral muscle (Krawczak, Kozłowska et al. 1988, Pieczynska, Wasik-Olejniak et al. 1985). The effect of dopamine on cutaneous blood flow has only been studied in infants of very low birth weight (Ishiguro, Suzuki et al. 2012). In these infants, the cutaneous and subcutaneous blood flow of the lower limbs was increased after dopamine treatment, supporting the findings of Eliassen et al. Therefore, in humans, dopamine could support the blood flow to peripheral skin and muscle tissues.

2.3.2 Circadian variation

The blood flow to the peripheral tissues is largely regulated by the autonomic nervous system (ANS), and especially its sympathetic branch (Doupe, Newman et al. 1939). The activity of ANS is divided into two major branches, the sympathetic and the parasympathetic nervous systems. The sympathetic-parasympathetic balance of the ANS has a circadian variation, seen as sympathetic domination during daytime and more parasympathetic tone during the night. This can be seen in blood pressure, cardiac output and most other measures of the ANS activity. It has been suggested that the parasympathetic branch of the ANS is predominantly under circadian influence, whereas the sleep-wake state controls the sympathetic activity (Burgess, Trinder et al. 1997). Oscillations in skin blood flow have been shown to be controlled predominantly by the sympathetic component (Soderstrom, Stefanovska et al. 2003).

There have been very few studies on the 24-hour circadian variation of blood circulation in the periphery due to the invasive or cumbersome nature of the measurement techniques. Veerman et al. studied the circadian variation of the systemic hemodynamics of the human body by measuring the blood pressure, heart rate, cardiac output and total peripheral resistance (Veerman, Imholz et al. 1995). They found that the total peripheral resistance showed a tonic increase during nighttime, whereas the arterial pressure and cardiac output decreases simultaneously. These parameters returned swiftly to normal after awakening in the morning. The authors concluded that the blood flow to the skeletal muscles is reduced during the night through means of local autoregulation, and returned back to the baseline level in the morning upon awakening. Similar results concerning the pattern of total peripheral resistance have been reported by other authors (Khatri, Freis 1967). Nocturnal increase of the total peripheral resistance has been also reported in non-human primates (Engel, Talan 1987), supporting these findings. However, contradictory results have been reported by one study. Bristow et al.

reported slightly decreased total peripheral resistance during the night time (Bristow, Honour et al. 1969). However, due to major differences in study design the result cannot be directly compared.

As a conclusion, although not well characterized, the blood flow to the periphery seems to be under circadian control in man. This is controlled both by the cardiac output and peripheral vasoconstriction and vasodilation, the sympathetic nervous system playing an important regulatory role.

2.3.3 Measurement

Many existing measurement techniques mirror the blood flow to peripheral tissues. These include laser-Doppler flowmetry, different kinds of photosensors, transcutaneous measurements of the partial pressures of blood gases and skin temperature measurements. In addition, finger photoplethysmography may be used to estimate the vasodilation and vasoconstriction in the extreme peripheries. This section will introduce all of them but focus on the methods used in this work.

2.3.3.1 Laser-Doppler flowmetry

Laser-Doppler flowmetry is based on a probe applied to the skin or other tissue, emitting light to the underlying tissue. The light will penetrate up to 2 millimeters into the skin. Blood cells flowing through the site of application will partially reflect the light resulting in a Doppler effect. The amount of Doppler-shifted light detected at the photodetector is therefore in direct relation to the quantity and velocity of the blood cells moving in the observed volume and an estimate of blood flow to that tissue may be computed (Riva 2001). If deeper tissues, for example the intramuscular blood flow in skeletal muscle, are of interest, a single-fiber technique may be used (Kvernebo, Staxrud et al. 1990).

The validity of laser-Doppler flowmetry measurements for assessing peripheral blood flow has been studied both in healthy human subjects and in special patient groups (Eun 1995, Kvernebo, Slagsvold et al. 1988). These studies have found laser-Doppler measurements to be a good estimate of blood flow to peripheral tissues. Another study observed the blood flow during sleep with the same method, concluding that it could be a good technique for assessing the function of the autonomic nervous system during sleep (Shiihara, Hirota et al. 1999). The technique has also been used in the context of peripheral vascular impairment (Terada, Miyai et al. 2007).

In addition to the measurements of skin blood flow, the technique of laser-Doppler flowmetry has been used in other contexts. The most common context is the measurement of blood flow in the eye (Riva 2001) and in the bone (Swiontkowski 1990). Laser-Doppler flowmetry has also been used in the context of RLS in one study assessing the peripheral blood flow (Anderson, Di Maria et al. 2013).

2.3.3.2 Skin temperature

Since one of the most important functions of the skin blood flow is thermoregulation, it could be assumed that recording of skin temperature reveals information about the fluctuations in the blood flow into the skin tissue. Blood transports heat from central parts of the body to the periphery, where it diffuses into the environment. The amount of heat transferred is naturally dependent on the quantity of blood flowing into the peripheral skin. Therefore, increased blood flow results in warmer skin and vice versa. The skin is easily accessible for temperature measurements, and thus a potential site for blood flow monitoring.

Skin temperature may be measured by thermal imaging or by probes attached directly to the skin. Thermal imaging may be performed with specialized cameras and may require calibration (Ring, Ammer 2012). With thermal imaging it is easy to measure the skin temperature of the whole body without attaching any probes to the subject. Skin temperature probes, on the other hand, are not practical when measuring whole body skin temperature, but are easier to use in long-lasting continuous measurements. These kinds of devices, although not originally developed for measurement of human skin temperature, have been validated for these measurements (van Marken Lichtenbelt, Daanen et al. 2006, Hasselberg, McMahon et al. 2011) and have been used in numerous studies for instance in the context of insomnia (Raymann, Swaab et al. 2007) or narcolepsy (Fronczek, Overeem et al. 2006).

Skin temperature measurements have been shown to correlate with blood flow to the skin tissue in studies with ambient temperature interventions (Bornmyr, Svensson et al. 1997). The blood flow was measured with laser-Doppler flowmetry. The same conclusion has been suggested by other studies (Krauchi, Cajochen et al. 2000). In more extreme conditions, such as rapid cooling of a hand, the relationship between skin temperature and skin blood flow has been shown to be non-linear (Vuksanovic, Sheppard et al. 2008). These results suggest that measurement of skin temperature gives a good estimate of the skin blood flow, but it is limited to normal ambient temperatures without sudden changes.

It is well known that the blood flow to the distal and proximal parts of the body are regulated by different systems (Boron, Boulpaep 2005). Therefore, it is of interest to record skin temperatures in both of these areas. With these measurements, the distal-to-proximal skin temperature gradient (DPG) may be computed to evaluate the distribution of blood between the central and peripheral parts of the body (Rubinstein, Sessler 1990). Shifts in DPG have been shown to correlate with sleep onset (Krauchi, Cajochen et al. 2000). The change coincides with increasing sleepiness and salivary melatonin, as well as decreasing core body temperature and heart rate (Krauchi, Cajochen et al. 2000), possibly marking the withdrawal of sympathetic influence upon falling asleep. The skin temperature shifts are also modulated by vigilance: reduced vigilance and increased sleepiness has been shown to elevate distal skin temperature, shifting it closer to the values of the temperatures of the proximal skin and core body (Romeijn, Van Someren 2011). In addition, in female subjects, the DPG is modulated by the menstrual cycle (Shechter, Boudreau et al. 2011).

In conclusion, the continuous monitoring of skin temperature gives a good estimate of skin blood flow, but may also be affected by the ambient temperature and various physiological factors. The estimation of blood flow may not, however, be applicable to underlying tissues, such as skeletal muscle, whose blood flow is regulated by different factor than that of the skin (Vissing 1997).

2.3.3.3 Transcutaneous gas measurements

The transcutaneous measurement of carbon dioxide (tcCO₂) and oxygen (tcO₂) are other potential methods for evaluating blood flow to the skin tissue. The methods themselves are old, but only recently have they been used in characterization of skin blood flow or the oxygen or carbon dioxide content of the skin tissue, for instance in overnight measurements (Aittokallio, Polo et al. 2008). These methods may be used when continuous and non-invasive monitoring of the partial pressures of carbon dioxide and oxygen in the skin tissue is of interest.

The measurement of tcCO₂ was developed in the 1970s after discovering that when skin was heated, the arterial partial pressure of oxygen could be estimated transcutaneously in newborn babies (Severinghaus 1998). Today, tcCO₂ and tcO₂ are measured with combined or separate sensors, based on the principles of the Clark pO₂ electrode and the Stow pCO₂ electrode (Severinghaus, Astrup et al. 1998). The sensors are attached onto the skin with an attachment ring. These sensors heat the skin to a predetermined temperature (often 43°C) in order to dilate the blood vessels in the skin. The heating of the skin has been shown to be

of great importance in order to get values relating to changes in blood flow to the skin tissue (Al-Siaidy, Hill 1979). This allows the gases to freely diffuse through the skin to be detected by the sensor through pH sensing (Eberhard 2007). The reading of the meter is then corrected by so called Severinghaus correction factor and metabolic correction factor in order to better estimate the arterial values of gas partial pressures at normal body temperature.

The tcCO_2 measurements have been used previously in the estimation of arterial partial pressure of carbon dioxide in newborn infants and in surgeries (Xue, Wu et al. 2010, Tingay, Stewart et al. 2005). For this reason, most studies have focused on comparing the readings of tcCO_2 to arterial values (Rosner, Hannhart et al. 1999, Janssens, Howarth-Frey et al. 1998). In addition to measurement of the arterial carbon dioxide content, tcCO_2 monitoring is also sensitive to changes in blood perfusion through vasoconstriction (Clark, Votteri et al. 1992). This makes the interpretation of the signal challenging, but also gives it a novel function: monitoring of blood perfusion of the skin tissue.

At higher probe temperatures, the level of tcCO_2 has been shown to correlate with measurements of skin blood flow (Christensen, Hjarbaek et al. 1991). It has been previously demonstrated that the level of tcCO_2 increases significantly upon transition from wakefulness to sleep (Chin, Hirai et al. 1997, Aittokallio, Virkki et al. 2006). This finding, also visible in measurements of skin temperature (Krauchi, Cajochen et al. 2000), could be a result of changing skin blood flow. In other sleep studies with adult subjects, tcCO_2 has been used in the characterization of the upper airway flow limitation during sleep (Rimpilä, Saaresranta et al. 2013) and during the therapy of obstructive sleep apnea with nasal CPAP (Fukui, Ohi et al. 1993). In addition, phenomena in nocturnal tcCO_2 have been associated with vascular impairment (Aittokallio, Polo et al. 2008) and metabolic risk factors (Aittokallio, Saaresranta et al. 2009). Despite these encouraging results, the method is far from being sufficiently validated for the measurement of skin blood flow and should be interpreted with caution.

Transcutaneous oxygen is measured with the same non-invasive principle as carbon dioxide: the skin under a sensor is heated and oxygen may diffuse through the skin to the sensor, thanks to the vasodilation of skin blood vessels. The sensor is based on the Clark sensor of the partial pressure of oxygen, typically combined with the Stow carbon dioxide electrode (Severinghaus, Astrup et al. 1998).

The measurement of tcO_2 , similarly to tcCO_2 , has been used most frequently in the continuous monitoring of newborn infants (Peevy, Hall 1985). In these subjects, the levels of tcO_2 have been characterized also during sleep (Garg,

Kurzner et al. 1988). For diagnostic purposes in adult populations, tcO_2 has been previously used in the diagnosis of vascular insufficiency (Byrne, Provan et al. 1984). Unfortunately, sleep studies in adults aiming at method validation or description, describing the levels of tcO_2 have not been reported in the literature.

The signal of tcO_2 has been slightly better characterized than the $tcCO_2$ signal in relation to skin blood flow. Early studies showed the correlation between the levels of tcO_2 and changes in the arterial blood pressure already in the 1980s (Eickhoff, Jacobsen 1980). Other studies have shown the close connection between fluctuations in skin blood flow and in the tcO_2 signal (Svedman, Holmberg et al. 1982, Braems, Lang et al. 1996). During hyperventilation, tcO_2 has been shown to be more responsive to skin blood flow and ventilation than the arterial partial pressure of oxygen (Barker, Hyatt et al. 1991). In addition, the levels of $tcCO_2$ have been shown to correlate with the oxygen content of the skeletal muscle underlying the skin, as demonstrated by MRI measurements (Partovi, Aschwanden et al. 2013). This could prove as another potential use of the tcO_2 signal.

In conclusion, the transcutaneous measurement of blood gases may be a good estimate of skin blood flow because of the influence of blood perfusion to the tissue. However, in the case of $tcCO_2$, the method has not been sufficiently validated for this purpose. Better validation studies exist for tcO_2 , showing good correlation with blood flow.

2.3.3.4 Finger plethysmography

Finger plethysmography continuously measures the volume of the pulse wave that travels from the heart to the tip of the finger. It may be performed by two different methods: by measuring the volume directly with volumetric probes (Grote, Zou et al. 2003), or optically with a light source and detector (Allen 2007). Optical measurement of finger plethysmography is most often coupled with an oximeter, measuring the arterial oxyhemoglobin saturation. Therefore, they are more commonly available in sleep laboratories and other hospital units. The major limitation of this method is the frequent saturation of the signal and the lack of viable calibration. Probes measuring the volume directly, on the other hand, do not have these problems. They are more reliable for recording finger plethysmography, but not as easily available in standard laboratories as the optical versions.

The most commonly used variable derived from the finger plethysmography signal is the pulse wave amplitude (PWA). The PWA method, also sometimes called the peripheral arterial tonometry, has been used in the study of peripheral

vascular endothelial function (Kuvin, Patel et al. 2003), suggesting it could be a potential method for studying peripheral vascular function. Temporary reductions in the PWA have been shown to be associated with arousals from sleep, most often related to respiratory events (Adler, Bridevaux et al. 2013, Delessert, Espa et al. 2010). These drops could be related to autonomic activations, also related to arousals from sleep (Pillar, Bar et al. 2002). Another study by Grote et al. assessed the response of PWA to pharmacological modulation of the sympathetic nervous system (Grote, Zou et al. 2003). They concluded that the responses in the PWA do not mirror the changes in forearm blood flow but are regulated independently. This discrepancy has been demonstrated in other studies (Longhurst, Capone et al. 1974). The principal regulatory system of the digital blood flow seemed to be the sympathetic nervous system. Therefore, the PWA could be a viable method for studying sympathetic impact on the blood flow to the periphery (Grote, Zou et al. 2003).

2.3.3.5 Other methods

Limb blood flow has been measured with various other methods in the past (Mowbray 1966). One of these methods is the measurement of clearance of an indicator from a tissue deposit. The clearance occurs as a function of blood flow to the tissue of interest, which may thus be measured. Volumetric methods include the plethysmography of whole arm or hand. The technique often includes venous occlusion in order to control the blood flow out of the tissue. In addition, thermal methods have been used for the same purpose (Mowbray 1966). These methods, however, include interventions that may themselves have an effect on the tissue blood flow. Therefore, using these methods is not rational when modern methods described above are available.

Other methods to assess blood flow include photosensors and thermal diffusion. Photosensors have been used in the measurement of blood flow also outside the optical finger plethysmography techniques. These techniques include the measurement of tissue hemoglobin, tissue blood volume and different properties of finger arteries (Nakamura 1997). Thermal diffusion, on the other hand, is a method measuring blood flow using a curve of thermal clearance. The problem with this method is that it cannot be measured continuously and the technique involves interventions potentially modulating the blood flow.

3 Aims of the present study

The specific aims of the study were to investigate:

- 1) The existence of hypoxia or hypercapnia in the peripheral skin tissue of patients suffering from RLS
- 2) The distal and proximal skin temperatures in restless legs syndrome and healthy controls
- 3) The effect of pramipexole medication on peripheral blood flow in RLS
- 4) The association of periodic leg movements during sleep with transient peripheral vasoconstriction
- 5) The association of periodic leg movements with cortical and autonomic arousals in complete spinal cord injury

4 Subjects and methods

The section is divided into two separate projects. Project 1, titled “peripheral hypoxia in RLS”, comprises of original publications I-III. Project 2, titled “PLM in spinal cord injury” is published as original publication IV.

4.1 Project 1: Peripheral hypoxia in RLS

4.1.1 Study subjects

Fifteen patients suffering from idiopathic RLS were recruited into the study from the patient registry of Unesta Research Centre. The patients were required to have a previous diagnosis of idiopathic RLS according to the official diagnostic criteria (Allen, Picchietti et al. 2003) and be currently on pramipexole treatment. The patients had to have plasma ferritin levels above 15µg/l in previous measurements, indicating no iron deficiency. All subjects with significant other medical conditions, such as neurological diseases, cardiovascular diseases or other sleep disorders, were excluded from the study. Only subjects more than 18 years old were included.

Fourteen control subjects were recruited primarily among the friends or colleagues of the patients, secondarily from other sources. The control subjects were matched for age and sex with the corresponding RLS patient. Control subjects were asked to report any RLS in the close family. If familial RLS existed, the subject was not included in the study.

A signed informed consent was obtained from all patients and control subjects before participation in the study. The study was approved by the local ethical committee in Tampere, Finland.

4.1.2 Study design

This observational study was executed as a case-control study. Discontinuation and subsequent re-continuation of pramipexole medication in the patient group

was the only medical intervention performed. The measurements lasted four days for each individual subject. Sleep habits and distal and proximal skin temperature were monitored for the whole duration of the study. The study protocol included four SIT sessions as well as two optional polygraphic sleep studies.

The potential patients and controls came to the screening visit at the sleep laboratory after being recruited to the study through a phone call. The purpose of the visit was to evaluate the eligibility of the patients and controls to the study. During the screening visit, the patients were interviewed by a physician with extensive experience in RLS diagnosis. The diagnosis of RLS was confirmed and an individual plan was made for each patient for discontinuation of current RLS medication. The pramipexole treatment was discontinued at least 14 days before the first day of measurements.

After the two-week washout of pramipexole, the patients and controls returned to the sleep laboratory for the first night of measurements. At the beginning of the visit, the skin temperature monitors were attached on the skin of all subjects. In addition, the severity of RLS and sleepiness during the discontinuation period were assessed with questionnaires. The standard IRLSSG questionnaire was used to assess RLS severity (Walters, LeBrocq et al. 2003), whereas sleepiness was evaluated with the Epworth Sleepiness Scale (ESS) questionnaire (Johns 1991).

Two SITs were performed during the first visit, two and four hours before the scheduled bedtime. After the SITs, the patients who were willing to participate in a polygraphic sleep recording, stayed at the sleep laboratory. The sleep recording was performed at the usual bedtime of the patient. In the morning after the sleep recording, the subjects were allowed to continue their daily life with only skin temperature monitoring. The patients who did not want to participate in sleep recording, were allowed to go home to sleep with only skin temperature monitoring.

During the second and third day of the measurements, the patients did not come to the sleep laboratory. Only skin temperature measurements and self-reported bedtimes were recorded from these two days. On day three, the subjects in the RLS patient group restarted their pramipexole medication. The dosage of pramipexole was planned individually, according to the dose the patient had used before and the dose that had been previously found to be effective. No higher daily doses than 0.50 µg were used. Control subjects were not asked to take pramipexole medication.

In the evening of the fourth day, the subjects returned to the sleep laboratory about five hours before bedtime. The SITs and optional sleep recordings from the

first evening were repeated. As the only difference to the previous tests, the RLS patients were asked to take their pramipexole medication about an hour before arriving to the sleep center for it to take effect before the start of the SIT sessions.

On the fifth day of the study, the patients were asked to remove the skin temperature measurement devices and return them to the sleep laboratory either personally or by mail. No other measurements were performed during the final day of the study. The protocol of the study is summarized in Table 1.

	Day -14	...	Day 1	Day 2	Day 3	Day 4	Day 5
Informed consent	X						
Screening and start of washout	X						
Installation of iButtons			X				
Questionnaires			X				X
Temperature monitoring			X	X	X	X	X
Sleep diary			X	X	X	X	X
Optional sleep recording			X			X	
Two SITs in the evening			X			X	
Pramipexole medication					X	X	X
Collection of iButtons							X

Table 1. The study protocol for the subjects in the RLS patient group. “X” indicates that the procedure is performed at the corresponding time.

4.1.2.1 Skin temperature measurements

Skin temperatures of the subjects were recorded during the entire 96-hour period the study lasted for each individual subject. The skin temperature recordings were performed using model DS1922L iButton devices (Maxim Integrated, San Jose, CA, U.S.). These devices have been validated for the use in evaluating human skin temperature (Hasselberg, McMahan et al. 2011), and they have been shown to have an accuracy of 0.09°C and precision of 0.05°C (van Marken Lichtenbelt, Daanen et al. 2006). The devices were set to record the momentary skin temperature every 120 seconds, while the resolution of the measurement was set at 0.0625°C. The devices were placed parasternally on the chest and on the instep of both feet, attached with adhesive tape. If detached during the study, the subjects

were asked to reattach the devices with the adhesive tape provided or with other methods.

In the afternoon of the fifth day, the patients were asked to remove the iButtons from their skin and return them to the sleep laboratory either personally or by mail. The data from the iButtons was then downloaded using OneWireViewer application (Maxim Integrated, San Jose, CA, U.S.) and imported to Microsoft Excel for further analysis.

4.1.2.2 Suggested immobilization tests (SIT)

In this study, SITs were performed according to a standardized protocol kindly provided by the Sleep Disorders Center of The Johns Hopkins Hospital in Baltimore, MD, USA. The SITs were performed in sleep laboratory conditions at the Unesta Research Centre, Tampere, Finland.

In previous literature, multiple SITs are recommended due to the fluctuation of RLS symptoms during the evening (Garcia-Borreguero, Kohnen et al. 2013). In our study, two SITs were performed per evening, approximately two and six hours before the scheduled bedtime. The most common times for performing the tests were 6 pm and 8 pm. Average values of the SITs were used in all analyses.

Before starting the SITs, all electrodes were attached to the subjects. During the SITs, the following parameters were measured: two channels of EEG (C3-Fz and C4-Fz), electrooculogram (EOG), electrocardiogram (EKG), submental and bilateral tibial electromyogram (EMG) and arterial oxy-hemoglobin saturation (SaO₂) with photoplethysmogram signal from the second toe of the right foot. Skin partial pressure of carbon dioxide (ptCO₂) and oxygen (ptO₂) were measured transcutaneously with TCM4 devices, coupled with Severinghaus type E5280 sensors (Radiometer, Copenhagen, Denmark). The transcutaneous measurements were performed both on the skin of the chest and the instep of the right foot. The place of the electrode was carefully chosen so that it was never placed on top of major veins or damaged or hardened skin. In addition to these measurements, also skin temperature was recorded during SITs in previously described manner. All data, except skin temperatures, was processed, stored and analyzed in the Somnologica Studio software (version 3.3.2., Medcare, Reykjavik, Iceland).

The SITs were performed in rooms with a bed, locked to a semi-sitting position. The lights were kept switched on during the entire tests. The ambient temperature was controlled in the rooms. The subjects were not allowed to read, watch television or do any other stimulating activity during the tests. Eyes were advised to be kept open and sleeping was not allowed. In order to control for bias

from individual clothing, all subjects had socks on during the tests, but no blankets. The tests were aborted if the patient felt symptoms that were intolerable and he or she could not remain immobilized for any reason. Between the two SITs, the subjects were allowed to walk and eat an evening snack.

The duration of each individual SIT was set at 60 minutes. During the test, the patients were asked every five minutes through a mobile radio telephone to report their momentary discomfort onto a questionnaire provided with in the beginning of the test. The scale of discomfort ranged from no discomfort (value 0) to maximal discomfort imaginable (value 10). The values were printed on the sheet and subjects were asked to circle the appropriate value at the time of the demand. The values of all other parameters of interest, including transcutaneous gas measurements, skin temperature and SaO₂, were recorded at the times when also the discomfort was demanded.

4.1.2.3 Sleep recordings

The optional sleep recordings related to this study were performed with a minimal setup, in order to minimize disturbance of sleep during the night. The setup included 2 channels of EEG (C3-Fz and C4-Fz), EOG, EKG, submental and bilateral tibial EMG and SaO₂ measured on the toe. The oximeter placed on the toe also continuously recorded the plethysmogram signal. The recordings were performed with similar setup during both nights at the sleep clinic.

The bedtime was set according to the habitual bedtime of the subjects. Each recording lasted for eight hours. No coffee was permitted during the last five hours before bedtime. No daytime naps were permitted during the study.

All recordings from SITs and polysomnography were performed with Embla N7000 sleep recording systems (Embla Systems, Broomfield, CO, USA) and processed and stored in the Somnologica Studio software (version 3.3.2., Medcare, Reykjavik, Iceland). Transcutaneous measurements were performed with TCM4 devices (Radiometer, Copenhagen, Denmark) with Severinghaus type E5280 sensors for both oxygen and carbon dioxide measurement. For further statistical analysis, the necessary data was exported to Microsoft Excel or SPSS.

4.1.3 Methods of analysis

The subjective discomfort, reported every 5 minutes during the SIT, was marked by the subjects on forms provided by the investigator. Values of other parameters of interest, including ptO₂, ptCO₂, skin temperatures and SaO₂, were

extracted as momentary values at the same time intervals as subjective discomfort. The distribution of oxygen in the body was evaluated by computing the gradient ptO_2 with the formula $ptO_2 \text{ gradient} = ptO_2(\text{chest}) - ptO_2(\text{foot})$. The gradient was computed in the same way for carbon dioxide measurements. Average levels of each parameter were computed from the two SITs for each time point.

The absolute levels and the gradients of the partial pressures in the legs were compared between the three different patient groups: patients without medication, patients with pramipexole medication and healthy controls. The measurements in different subjects were compared in individual time points during SIT, as well as average values during the whole SIT.

Correlations of the measured parameters with RLS severity, measured by the IRLSSG scale, were computed. RLS patients who did not have high enough disease severity (IRLSSG severity < 15 , $n = 3$) at the start of the study were excluded from the analyses comparing RLS patients and controls but not from the analysis of correlation between RLS severity and measured values.

Values of skin temperature were extracted with the interval of 5 minutes during the SITs. Average values of the skin temperatures in the two tests were used in the analyses. Skin temperature was analyzed both as the average peripheral skin temperature (mean of the temperature of the two feet) and as distal-to-proximal skin temperature gradient ($DPG = T(\text{foot}) - T(\text{chest})$).

Daytime temperatures were analyzed in time periods of one hour. The different recordings were fixed to a timeframe according to the time of self-reported awakening in the mornings. The analysis started two hours before the awakening in the morning, the first time window lasting from two to one hour before the awakening. The analysis continued in similar one-hour time blocks until ten hours after the awakening. In each time block, an average value of the skin temperature was computed from the one-hour period. This mean value of the different days was used in the analyses of skin temperature at each time point.

The sleep recordings were used in analysis of PLM. The sleep states were analyzed in all sleep recordings in 30-second epochs according to the standard AASM criteria (Walters, Lavigne et al. 2007). Leg movements during the night were identified using the standard criteria established by the WASM task force in 2006 (Zucconi, Ferri et al. 2006). Movements were marked both during wakefulness and sleep.

After analysis of sleep states and PLM, further filtering of PLM events was applied to the data. PLM events that were occurring during or close by to desaturations of the SaO_2 , were interpreted as apnea-related leg jerks and were

excluded from the analyses. In addition, movements that happened during EMG, SaO₂ or plethysmogram signals of insufficient quality were not accepted. In addition, movements that were too close to other leg jerks, happening within 10 seconds before or 20 seconds after the movement, were excluded.

The plethysmogram data and time stamps of leg movement events were extracted from Somnologica Studio to text files. For analyzing these data, a custom algorithm was created using the programming language R. After reading the plethysmogram data, the data was segmented with help of the PLM time stamps. A time window of 30 seconds (from 10 seconds before to 20 seconds after the start of the movement) was identified for each leg movement. For each sentence, a heart rate level was analyzed by calculating spectral density. The heart rate was used to calculate a running average of the plethysmogram signal in each segment. The data was then further segmented to data below and data above the running average. These segments were used to identify local maximums and minimums in the data. Maximum and minimum values were discarded as movement artefact if they did not match with the corresponding running maximum or minimum. After obtaining the peaks and nadirs in the signal, representing the pulse wave, two separate envelope curves were created through these points. An example of the creation of the envelope curve is displayed in Figure 2.

After the creation of envelope curves for each individual leg movement event, collective values of all events of each patient were calculated. The analysis was done separately for events during wakefulness and sleep. The 30-second segments were divided into 14 time points, with two-second intervals. In these time points, the median of the values of all envelope curves was calculated. This was done separately for the two envelope curves, representing the maximums and minimums of the pulse wave. The medians were displayed then together with the first and third quartiles for each patient during wakefulness and sleep.

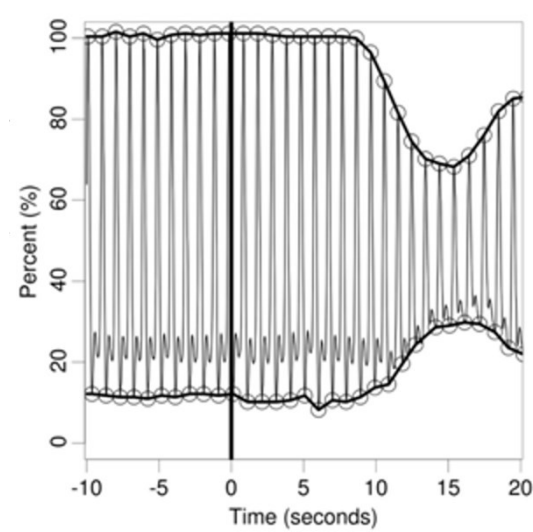


Figure 2. An example of the creation of the envelope curve for a single event. The detected peaks and nadirs are represented by circles, whereas the two envelope curves are drawn with black lines. The y-axis is shown as percentage of the technical maximum of the original units of the photoplethysmograph signal. On the x-axis, the start of the leg movement is shown as zero value and a vertical line.

4.1.4 Statistical methods

All statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) or SPSS software (IBM Corporation, Endicott, NY, USA).

Three primary hypotheses were tested for both the oxygen and carbon dioxide measurements:

1. Peripheral partial pressures are altered in patients with RLS compared to controls
2. The measured partial pressures show correlation with IRLSSG score of RLS severity
3. Peripheral levels are modified by pramipexole treatment in RLS patients.

Carbon dioxide and oxygen values were compared between the two groups at each time point during the SIT with two-tailed Mann-Whitney test. RLS patients with and without medication were compared with two-tailed Wilcoxon signed-rank test. Spearman's rank correlation coefficient was used for the evaluation of all correlations.

Skin temperatures during the SIT and during daytime were compared between the subject groups. Skin temperatures between patients without medicine and controls were compared at each time point with two-tailed Mann-Whitney test, both during daytime and SIT. Skin temperature levels of RLS patients with and without medication were compared with two-tailed Wilcoxon signed-rank test. Correlations with RLS severity were evaluated with Spearman's rank correlation coefficient. All subjects were included in the analysis of correlation.

4.2 Project 2: PLM in Spinal Cord Injury

4.2.1 Case report

A 35-year-old Caucasian male, with a spinal cord injury (SCI) due to a car accident at the age of 18 years, underwent a polygraphic sleep study performed in the context of a larger study focusing on neuroimaging and respiratory and urologic functions in patients with cervical SCI.

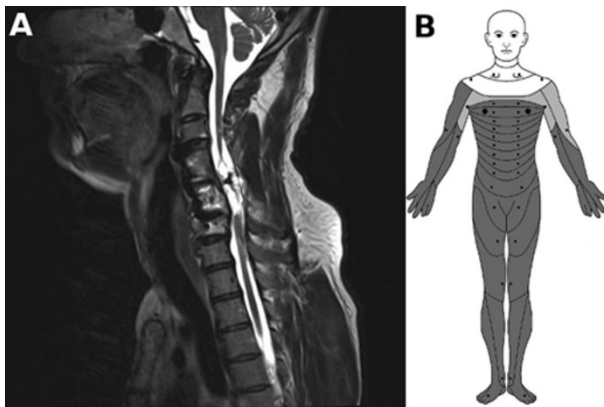


Figure 3. The injury of the patient is demonstrated by MRI (A) and classification of sensory function (B). Dark gray areas represent no sensory function, lighter gray represents partial sensory function and white areas were found to have full sensory function.

Previously performed 3-Tesla magnetic resonance imaging (MRI) showed the complete cervical spinal lesion between C3 and C5 (Figure 3). A classification of the SCI had been performed according to The International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) guidelines (Waring, Biering-Sorensen et al. 2010). The injury was complete, indicating ASIA (American Spinal Injury Association) impairment scale severity A (ASIA A). The patient had

no motor and very little sensory function below the C5 level (Figure 3). At the moment of the sleep studies, the patient was treated with tizanidine (12 mg daily) and baclofen (30 mg daily) for spasticity and nitrofurantoin (75 mg daily) for prevention of urinary tract infection. No other concomitant medication was taken during the studies, including analgesics. The patient reported neither sensory symptoms suggesting RLS nor daytime sleepiness (Epworth sleepiness score 2/24).

4.2.2 Measurements

The first baseline polysomnography included the continuous recording of EEG, EOG, EKG, submental and tibial bilateral EMG, SaO₂ and the measurement of breathing through nasal cannula and respiratory inductance belts attached around the abdomen and chest. This standard polygraphic sleep recording was carried out in sleep laboratory environment.

Thirteen months later, three additional sleep recordings were performed during three consecutive nights. During the first night, the baseline recording was repeated with similar settings. During the next night, the patient was given a single dose of pramipexole (0.25 mg, Orion Pharma, Espoo, Finland) two hours before bedtime, in order to assess the response of the PLM to dopaminergic medication. During the fourth sleep recording, the patient's sleep apnea was treated with adaptive servoventilation. No pramipexole was given before the last night. The second baseline recording and subsequent recordings with interventions were performed at the patient's home due to the reduced mobility of the patient. However, the same devices were used as in the first baseline recording.

The study was approved by the local ethical committee. Written informed consent was obtained from the patient.

4.2.3 Methods of analysis

In the sleep recordings, the sleep and breathing were scored in 30-second epochs according to the standardized method. The PLM were analyzed in each recording with previously described methods (Ferri, Zucconi et al. 2006). This method allows the construction of distribution curves of inter-movement intervals, used to evaluate the type of movements (Ferri, Zucconi et al. 2006). Average heart rates, relative to the baseline heart rate, were plotted in a manner used in previous studies (Ferri, Zucconi et al. 2007). In order to assess the heart rate response to the PLM, the start of each movement was used as a marker to fix the time window.

Additionally, a similar plot of average heart rates was made for arousals unrelated to PLM, in order to assess the heart rate response to those arousals.

5 Results

5.1 Project 1: Peripheral hypoxia in RLS

5.1.1 Demographics

The mean ages in the RLS patient and control groups were 57.1 and 56.6 years, respectively. In the RLS group, 7/15 subjects were female, whereas in the control group the number of women was 6/14. The mean RLS severity at the start of the study, when off medication, was 23.7 in the RLS patient group on the IRLSSG severity scale. There was no difference in sleepiness between the patients and controls (ESS score 6.1 vs. 4.3, $p=0.275$). One subject in each subject group had to be excluded from the analyses because of insufficient quality of the transcutaneous recording.

A total of ten patient and ten controls underwent the polysomnography. In this subgroup of patients, seven patients and seven controls were male. The average age in this group of subjects was 56.6 years for the RLS patients and 55.1 years for the controls. The mean RLS severity score in the patient group was 22.5/40.

5.1.2 Subjective discomfort during SIT

Subjective discomfort, prompted every 5 minutes during SIT, showed a great difference between RLS patients and controls (4.2/10 vs. 0.1/10, $p<0.001$). The discomfort was partly resolved by the continuation of pramipexole medication (from 4.2/10 to 1.6/10, $p<0.001$). Figure 4 illustrates the trend of discomfort during SIT in each patient group. The patients with RLS suffered from greater discomfort in the end of the immobilization, compared to the beginning.

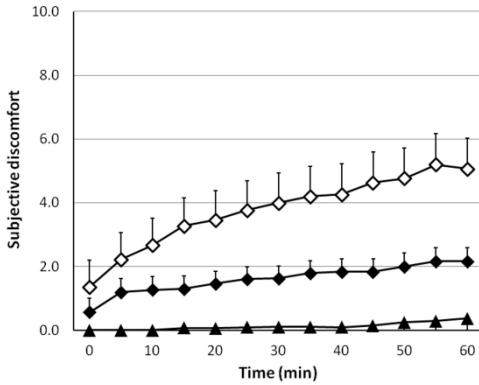


Figure 4. The evolution of subjective discomfort during SIT is displayed in different subject groups. Subject groups: RLS patients without medication (◇), RLS patients after pramipexole medication (◇) and healthy controls (▲). * $p < 0.05$ compared to RLS group without therapy.

5.1.3 Peripheral partial pressure of oxygen during SIT

There was no difference between the two groups in central ptO_2 levels on the chest (8.75 kPa vs. 8.20 kPa, $p = 0.355$). On the other hand, RLS patients had lower oxygen levels in their legs during the SIT compared to controls (5.54 kPa vs. 7.19 kPa, $p < 0.01$). Also the chest-to-foot ptO_2 gradient differed significantly between the two groups (3.22 kPa vs. 0.93 kPa, $p < 0.01$).

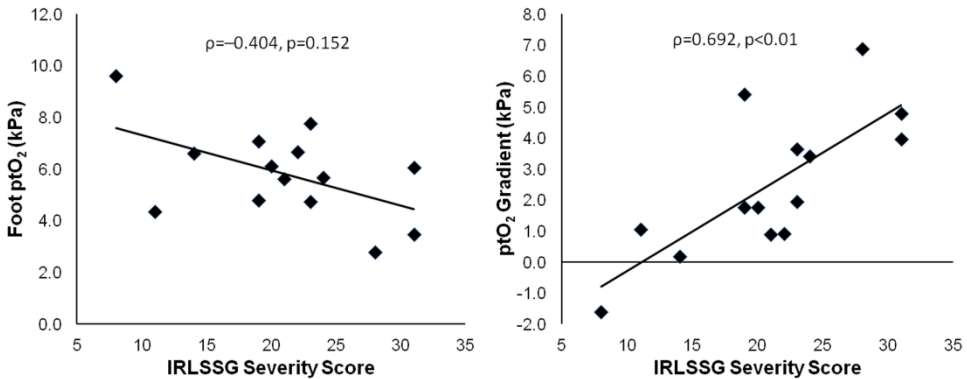


Figure 5. The values of foot partial pressure of oxygen (ptO_2) and the oxygen gradient in each patient during SIT are displayed in a scatter plot with RLS severity, measured by the IRLSSG questionnaire.

The ptO_2 gradient had a strong, statistically significant, correlation with RLS severity: the more severe RLS the patient was suffering from, the greater the difference in ptO_2 was between the chest and the periphery (Spearman's correlation $\rho=0.692$, $p<0.01$). Foot ptO_2 did not show a statistically significant correlation with RLS severity ($\rho=-0.404$, $p=0.152$). Scatter plots of these parameters, plotted against the disease severity, are displayed in Figure 5.

The commencement of pramipexole medication did not have a statistically significant effect on ptO_2 on the chest (from 8.75 kPa to 7.86 kPa, $p=0.156$). On the other hand, it increased the foot ptO_2 (from 5.54 kPa to 6.65 kPa, $p<0.05$) and consequently decreased ptO_2 gradient (from 3.22 kPa to 1.21 kPa, $p<0.01$) during SIT. Figure 6 displays the effect of pramipexole on the evolution of different oxygen parameters during the SIT. Although the effect of pramipexole on foot ptO_2 was significant when comparing mean values during SIT, it was not statistically significant at every time point.

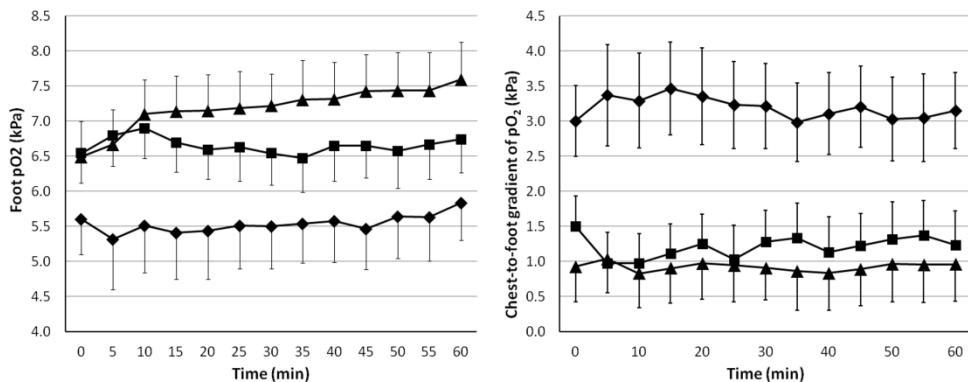


Figure 6. The evolution of foot partial pressure of oxygen (ptO_2) and the oxygen gradient during SIT is displayed in each group of subjects. The subject groups are RLS patients without medication (◇), RLS patients after pramipexole medication (◆) and healthy controls (▲). * $p<0.05$ compared to RLS group without therapy.

In the most severe RLS cases ($n=3$, IRLSSG severity between 28 and 31), the patterns of leg movements and foot ptO_2 were visually interconnected during SIT, as shown in the example in Figure 7: ptO_2 was declining spontaneously when legs were relaxed. The oxygen level increased momentarily when movements occurred, and decreased again after subsequent relaxation. This pattern was specific to the most severe cases of RLS.

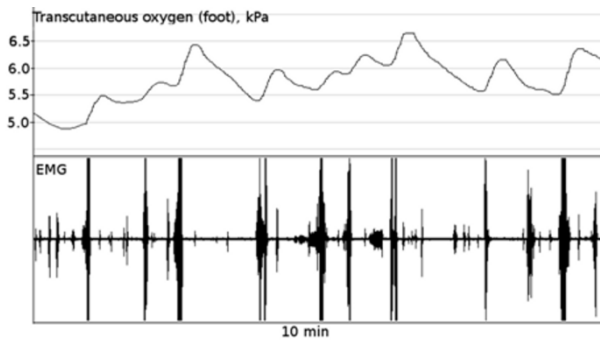


Figure 7. An extract from the SIT recording of a female patient with severe RLS. The example shows the closely related pattern of leg movements and the partial pressure of oxygen on the leg. The patient was reporting maximal discomfort at the time (10/10).

Arterial oxyhemoglobin saturation, on the other hand, did not show any difference between the patient groups (97.7% vs. 97.7%, $p=0.955$). Pramipexole did not change the level of SaO_2 in the patients suffering from RLS (from 97.7% to 97.5%, $p=0.477$). These measurements did not correlate with the severity of RLS.

5.1.4 Peripheral partial pressure of carbon dioxide during SIT

No difference was found between the patients and controls in carbon dioxide measurements on the foot (6.82 kPa vs. 6.92 kPa, $p=0.955$) or in the $ptCO_2$ gradient (-0.12 kPa vs. -0.11 kPa, $p=0.691$). However, the more serious cases of RLS also had higher levels of absolute $ptCO_2$ in their legs (Spearman's correlation $\rho=0.624$, $p<0.05$). The $ptCO_2$ gradient did not show statistically significant correlation with the RLS severity ($\rho=-0.463$, $p=0.095$). Figure 8 displays scatter plots of the carbon dioxide parameters plotted against RLS severity.

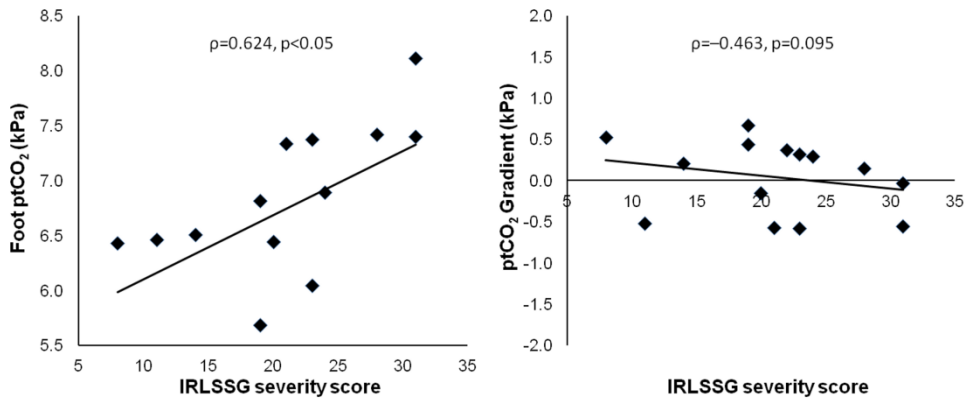


Figure 8. The values of foot partial pressure of carbon dioxide (ptCO₂) and the oxygen gradient in each patient during SIT are displayed in a scatter plot with RLS severity.

Pramipexole treatment did not significantly alter the mean carbon dioxide levels on the foot (from 6.82 kPa to 7.24 kPa, $p=0.173$) or ptCO₂ gradient (from -0.12 kPa to -0.04 kPa, $p=0.477$). Figure 9 shows the evolution of carbon dioxide in different patient groups during SIT, as well as the effect of pramipexole. Even if the effect of pramipexole medication on mean ptCO₂ on the foot was not significant during SIT, the effect was statistically significant at individual time points at the end of the immobilization.

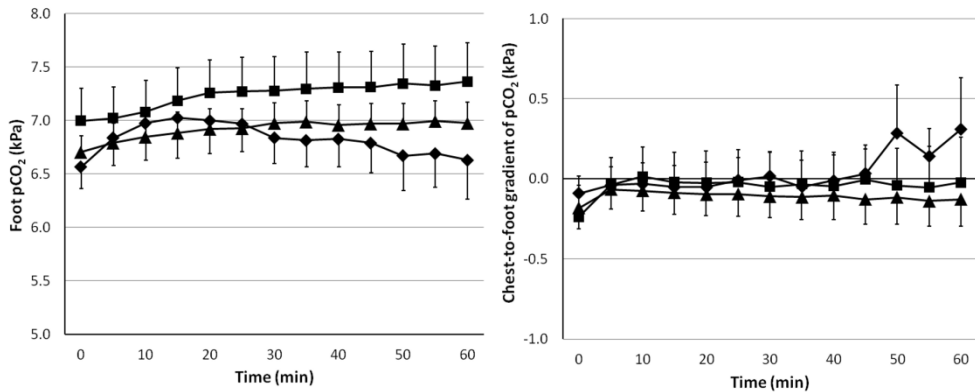


Figure 9. The evolution of foot partial pressure of carbon dioxide (ptCO₂) and the oxygen gradient during SIT is displayed in each group of subjects. The subject groups are RLS patients without medication (◇), RLS patients after pramipexole medication (◆) and healthy controls (▲). * $p<0.05$ compared to RLS group without therapy.

5.1.5 Skin temperatures in RLS

The distal skin temperatures did not differ between the patient and control groups (31.1°C vs. 31.1°C, $p=0.991$). No difference was found in the distal-to-proximal temperature gradient either between the two subject groups (-3.1°C vs. -3.0°C, $p=0.916$). Pramipexole, on the other hand, increased the skin temperature in the extremities but not on the chest. This can be seen as a rise in the distal skin temperature (from 31.1°C to 32.9°C, $p<0.05$) and DPG (from -3.1°C to -1.3°C, $p<0.05$). Average skin temperature values did not correlate with RLS severity (Foot temperature; $\rho = -0.110$, DPG; $\rho = -0.221$). Figure 10 displays the evolution of foot skin temperature and DPG during SIT in the different patient groups.

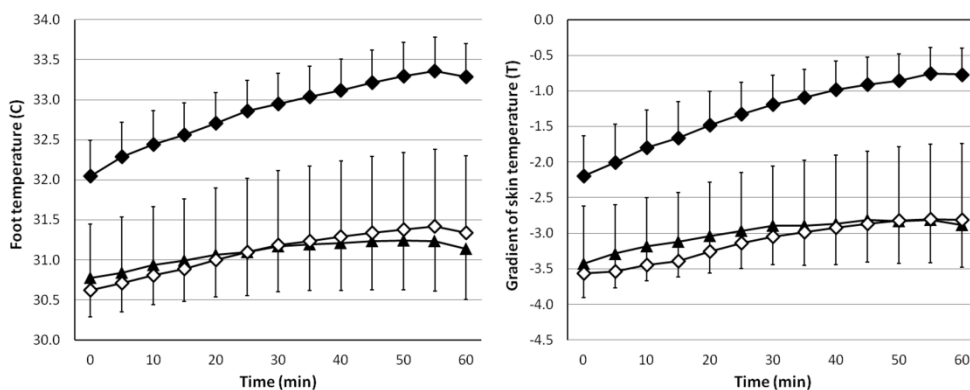


Figure 10. The evolution of foot skin temperature and the temperature gradient during SIT is displayed in each group of subjects. The subject groups are RLS patients without medication (◇), RLS patients after pramipexole medication (◆) and healthy controls (▲). * $p<0.05$ compared to RLS group without therapy.

The 96-hour continuous skin temperature measurements revealed high distal skin temperatures during the night, close to the level of normal core body temperature. During the first hours of wakefulness, the temperature dropped drastically. Therefore, during the day the average temperatures were 4-5°C lower than the nocturnal values. Consequently, the DPG was close to zero during the night and dropped during daytime.

No differences were observed between RLS patients and healthy controls in these measurements, indicating similar daytime skin temperatures. Pramipexole did not have an effect on the skin temperature of the following day. The daytime pattern of skin temperatures in different subject groups can be viewed in Figure 11.

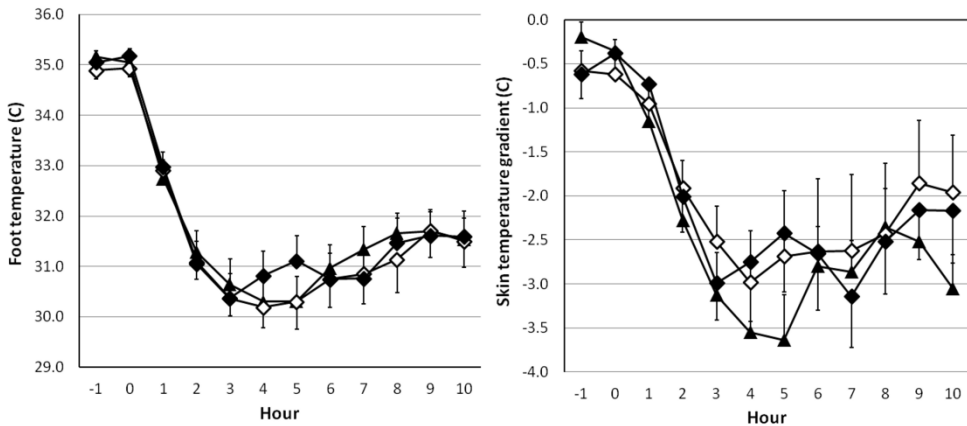


Figure 11. The evolution of foot skin temperature and the temperature gradient in daytime measurements is displayed in each group of subjects. The subject groups are RLS patients without medication (◇), RLS patients after pramipexole medication (◆) and healthy controls (▲). *p<0.05 compared to RLS group without therapy.

5.1.6 Peripheral vasoconstriction related to PLM

A total of 2606 leg movements were included in the final analyses in this study. Of these movements, 2090 (80%) occurred during sleep whereas 516 (20%) were identified during wakefulness. A total of 809 leg movements (24% of all movements detected) were identified but not analyzed because of insufficient signal quality. In most cases, this was due to difficult movement artefact or complete absence of signal during sensor failure. The number of leg movements included into the study varied from zero to 808 per patient (Table 2). One patient did not have leg movements in the PSG performed.

Patient ID	Sleep	Wakefulness	Total
1	371	153	524
2	221	20	241
3	0	0	0
4	224	73	297
5	120	52	172
6	693	115	808
7	248	34	282
8	99	11	110
9	41	40	81
10	73	18	91
Total	2090	516	2606

Table 2. The number of periodic leg movement events in each patient during sleep and wakefulness.

The responses in the pulse wave amplitude (PWA) to the PLM during sleep were different from patient to patient. Four out of ten patients had no responses in the plethysmography signal that were consistently temporally coupled with the PLM. Four patients, on the other hand, had clear visual dips in the pulse wave amplitude when the leg movements occurred. In these four patients, the PWA showed an average drop of 24% (range 12-30%) from the baseline level prior to the movement during sleep. During wakefulness, on the other hand, the drop was slightly lower 12% (range 6-20%) from baseline. In cases of single movements, the dip in the PWA could be up to 50% from baseline. The dip in the PWA did not start prior to the start of the leg movement, but 2-10 seconds after the movement. The dip lasted for 10-20 seconds, after which the PWA rose back to the baseline level. One recording patient had a saturated photoplethysmogram signal, which made the evaluation of PWA dips impossible. Figure 12 shows the average PWA dips related to leg movements in two patients with different responses.

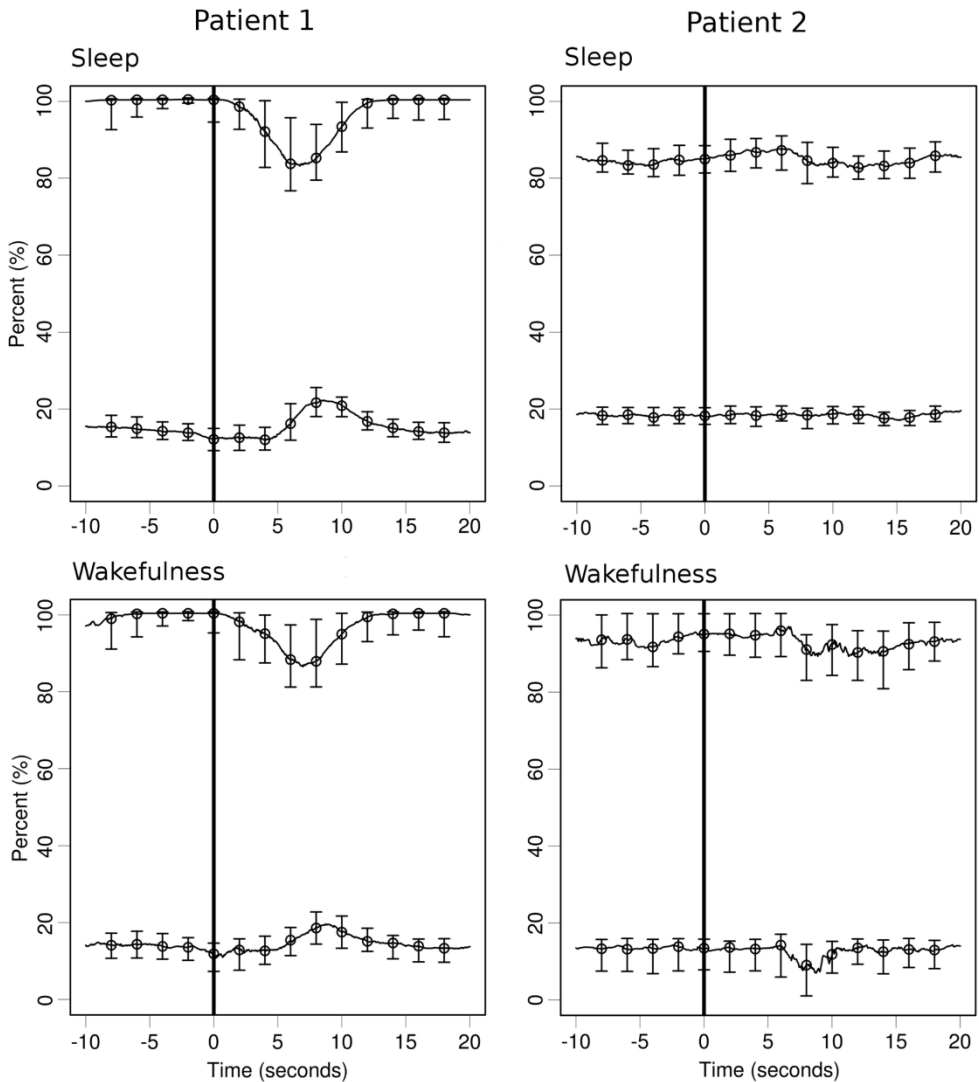


Figure 12. The PWA responses of two patients to PLM are displayed. The y-axis is shown as percentage of the technical maximum of the signal. On the x-axis, the start of the leg movement is marked with the value zero and a vertical line.

If a patient had transient responses in the PWA to PLM during sleep, the same patient also had responses during wakefulness. However, the magnitude of these responses was often smaller during wakefulness than during sleep. Then again, if a patient had no responses during sleep, the responses were absent also during wakefulness.

The continuation of pramipexole medication suppressed the leg movements. Cortical arousals remained unaffected by the therapy. However, these arousals were not coupled with PWA dips anymore, suggesting that pramipexole also suppressed the transient vasoconstrictions (Figure 13).

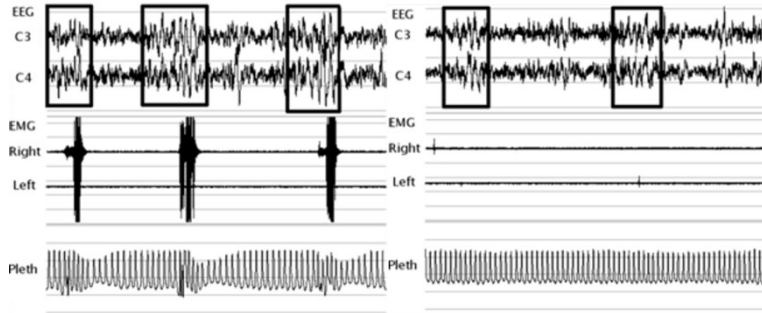


Figure 13. The effect of pramipexole on leg movements, cortical arousals and dips in pulse wave amplitude is demonstrated by examples from two sleep recordings of the same patient. In the first recording (left panel), the patient had leg movements with associated cortical arousals and transient vasoconstriction. In the second recording, after the continuation of pramipexole, the movements and vasoconstrictions were suppressed (right panel). EEG = electroencephalogram, EMG = electromyogram, Pleth = photoplethysmogram.

5.2 Project 2: PLM in Spinal Cord Injury

The PLM index was 138/h in the first sleep recording of the patient with complete spinal cord injury. The appearance of PLM was confirmed in a second polysomnography (PLM index 36/h), performed 13 months later. In addition, a high number of events of obstructive sleep apnea were found (apnea-hypopnea index of 45.5/h and 74.5/h), alongside with paradoxical breathing due to the paralysis of the intercostal muscles.

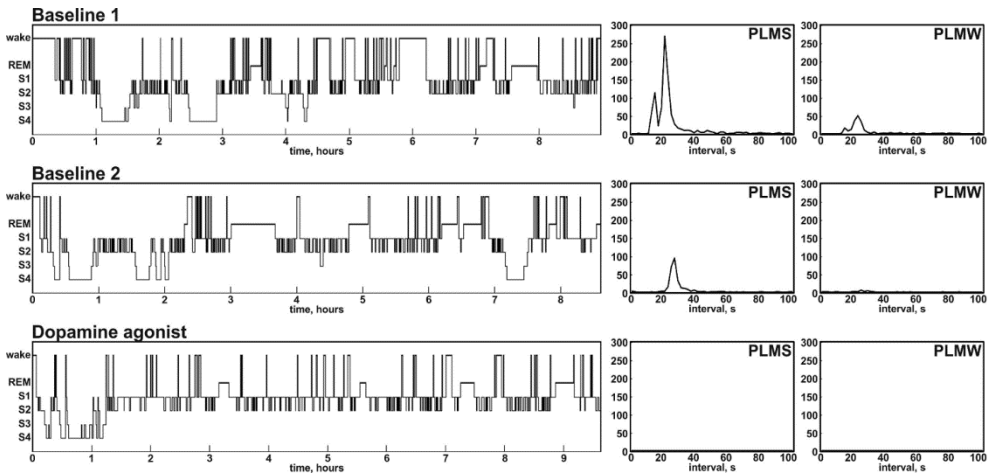


Figure 14. The hypnograms and inter-movement interval distributions of PLM are displayed for each of the three first study nights. PLMS = periodic leg movements during sleep, PLMW = periodic leg movements during wakefulness.

PLM occurred in a highly stereotypic pattern, unaffected by changes in state of vigilance or sleep stage (wakefulness, N1-N3 or REM sleep). They were present exclusively in the right leg, and fulfilled the standard scoring criteria for PLM. The interval distribution of leg movements showed a leptokurtic peak at 20-30 seconds in both baseline recordings (Figure 14). The same peak was present in the first baseline study during wakefulness. A peak normally occurring between 3 and 5 seconds, outside of the PLM range in patients with RLS, was not identified in this patient.

Respiratory events and related cortical arousals and heart rate elevations were synchronized, but consistently temporally dissociated from the leg movements (Figure 15). Heart rate elevations were associated with the cortical arousals, but were absent before or after the PLM (Figure 16). Resumption of breathing, accompanied by cortical and autonomic arousals at the end of the events of sleep apnea, was not associated with leg movements or jerks.

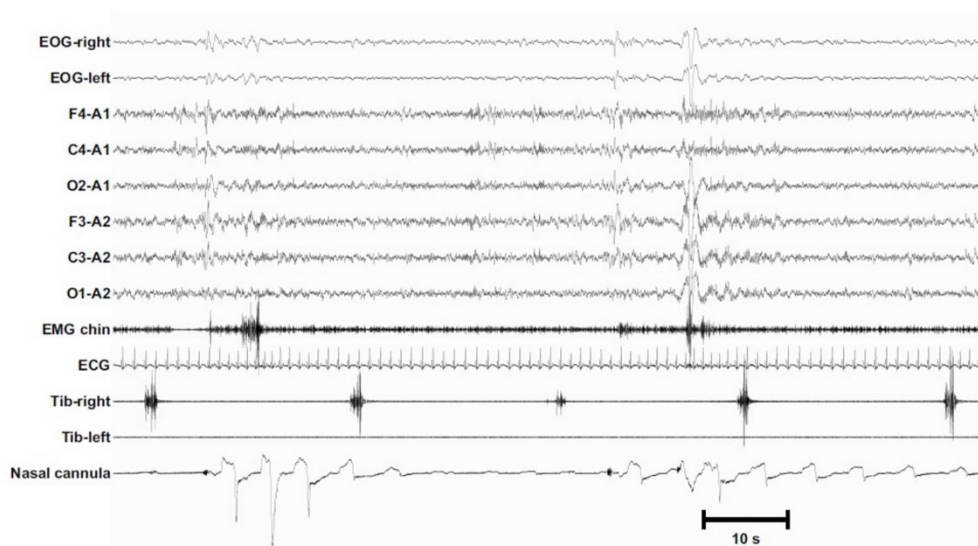


Figure 15. An extract of the first sleep recording shows the temporal disconnection between cortical arousals and periodic leg movements.

The single dose of pramipexole abolished PLM, while sleep apnea and related arousals remained unaffected. Leg movements appeared again during the following night when pramipexole was discontinued and sleep apnea was treated with adaptive servoventilation.

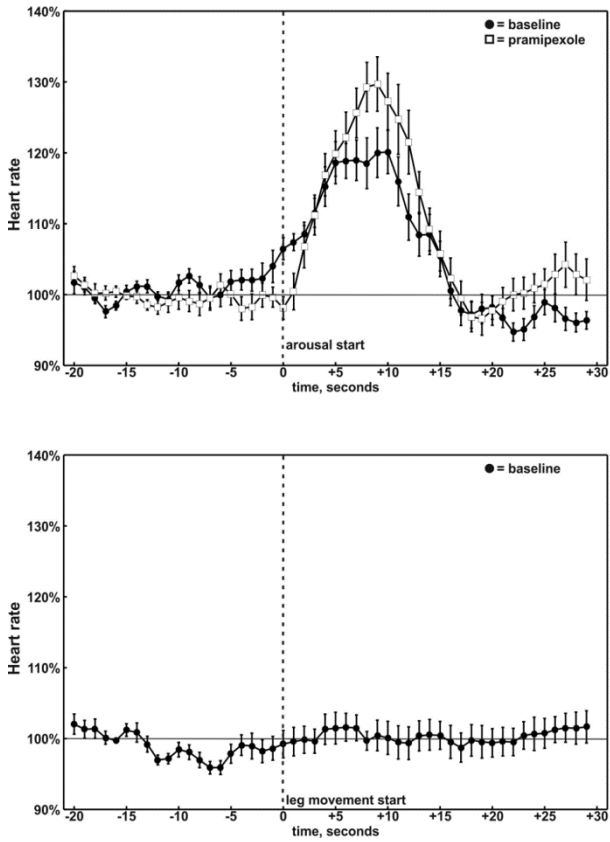


Figure 16. Average heart rate responses to cortical arousals and leg movements are displayed. Arousals not related to PLM were accompanied by elevations in heart rate (upper panel). Heart rate elevations, however, were not associated with the start of PLMs (lower panel).

6 Discussion

The primary purpose of this work was to test the theory of reduced peripheral blood flow in RLS through direct measurements of oxygen, carbon dioxide and skin temperature in the legs of RLS patients. Prior to these studies, only indirect evidence had been presented for the existence of hypoxia in the peripheral tissues of patients with RLS. Due to the lack of evidence, the theory of peripheral involvement in the pathogenesis of RLS had stayed without further scrutiny. In this work it was shown that peripheral hypoxia develops during the symptomatic period in patients with RLS. This hypoxia is reversible with pramipexole, a dopamine agonist. Therefore, hypoxia may have a more central role in the generation of RLS symptoms than previously thought.

The secondary purpose was to find further evidence of the peripheral involvement in the pathogenesis of PLM. The involvement of the autonomic nervous system is well established in relation to PLM, shown by transient elevations in heart rate and blood pressure (Ferri, Zucconi et al. 2007). However, the generation of the movements has been thought to occur in the brain (Montplaisir, Lorrain et al. 1991). Here we show that the movements may occur independently from the brain, and that the movement-related sympathetic activation also involves peripheral vasoconstriction.

6.1 Methodology

6.1.1 Subjects

The selection of subjects and controls was carefully controlled in the studies. Major confounding factors for peripheral blood flow, such as age and gender, were controlled for. The selection of control subjects was done through the corresponding patient's own close network in order to find subjects with similar environment and habits. Close relatives of RLS patients were not accepted and detailed interviews were performed to exclude RLS in control patients. All selection bias could not be addressed with this inclusion method. It is possible that

the control subjects recruited by our method did not represent the healthy general population. However, it was carefully controlled that the controls were demographically as close to the corresponding RLS patients as possible.

The results may be generalized to patient populations with moderate to severe idiopathic RLS. However, due to the reasonably small sample size, the results need to be confirmed in future studies with larger populations. Caution should be applied when generalizing the results to patients with uncommon types of RLS or one of its secondary forms.

6.1.2 Study protocol

There are several potential sources of bias arising from the study design in a study like this one. The measurement of skin temperature and the transcutaneous gas measurements could be severely biased by environmental conditions, such as ambient temperature. This kind of bias was addressed with study design. The patients and corresponding control subjects were attending the SITs as well as the polysomnography always at the same time and in the same place. This way, if potential bias was present from ambient temperature or other environmental factors, it concerned both groups of subjects. In addition, the air temperature in the rooms was controlled by an automatic system and the clothing of the patients at the sites of attachment was controlled by the investigator during SIT.

The effect of pramipexole could be affected in different patients by previous use of the same therapy or the dose and time of administration. All patients in our study were previous users of pramipexole. Therefore, all patients were affected by the previous usage of pramipexole in the same way. Time of administration was controlled by the investigator, and was always at least 90 minutes before the start of the first SIT. The dose of pramipexole was decided individually to the previously tested lowest effective dose. However, in order to minimize variation, no doses lower than 0.25 mg or higher than 0.5 mg were used in the study.

Some potential sources of bias exist that could not be controlled for by the study protocol. These sources of bias concern mainly the daytime skin temperature measurements, although some could introduce bias also to the SITs. The most evident factor is the weather and ambient temperature during the 96-hour temperature recording. The patients spent most of that time at home, and therefore the environment could not be controlled during that time. However, the effects of the time of year could be addressed through simultaneous recording of patients with their corresponding control subjects.

Another potential confounder could be the individual drowsiness during both the SITs and skin temperature measurements. Drowsiness could affect at least the skin temperature measurements (Romeijn, Van Someren 2011), possibly also the transcutaneous measurements. Napping was forbidden during the recordings, but there was no way or making sure that the patients did not sleep during daytime while at home. Sleep hours were controlled during the recording but individual stress or fatigue could have affected the measurements. In future studies, a constant routine protocol (Czeisler, Allan et al. 1986), designed to evaluate human circadian profile, should be applied to control for these factors increasing bias.

6.1.3 Transcutaneous measurements

Arterial oxygenation is conventionally measured by pulse oximetry, estimating the level of SaO₂. This measurement gives a good estimation of the oxygen bound to blood hemoglobin in the blood, especially during sleep apnea or hypoventilation. However, the SaO₂ measurement is not sufficient to tell how much oxygen the target tissue receives per minute, since this is also controlled by local blood perfusion. The levels of ptO₂ and ptCO₂ are, on the other hand, affected by blood perfusion, and are therefore sensitive to vasodilatation and vasoconstriction (Healey, Fedullo et al. 1987). Non-invasive methods have been developed to estimate the levels of ptO₂ and ptCO₂ in skin tissue. Among these methods, transcutaneous measurements offer the most flexibility in terms of mobility and continuous recording.

In this study, we used transcutaneous measurements to measure ptO₂ and ptCO₂ in the skin tissue. The validation of these methods for the measurement of tissue oxygen and carbon dioxide is somewhat insufficient and challenging in the absence of appropriate reference methods, but they have been used for similar purposes before. TcO₂ has been used in the diagnosis of peripheral vascular insufficiency (Byrne, Provan et al. 1984), and agrees well with MRI measurements of oxygen in certain patient groups (Partovi, Aschwanden et al. 2013). TcCO₂, on the other hand, is often used in surgery to evaluate limb ischemia (Sugimoto, Ohta et al. 2009). These studies give indication that these methods could be used in the measurement of ptO₂ and ptCO₂. However, further validation is needed to determine which phenomena affect these readings and what kinds of interventions they respond to. Nevertheless, we are convinced that, although not fully validated, the tcO₂ and tcCO₂ are the most appropriate methods to non-invasively and continuously monitor ptO₂ and ptCO₂ in the skin tissue.

In our study, we used the transcutaneous sensors heated to 43°C. This sensor temperature was chosen based on previous experience and literature. It has been shown that the temperature of 43°C is required for reliable readings in both oxygen and carbon dioxide measurements (Wimberley, Gronlund Pedersen et al. 1985). The Severinghaus correction of the oxygen and carbon dioxide reading was turned off during the recordings. This was done in order to directly measure the partial pressures of the gases in the tissue instead of trying to estimate the corresponding arterial values.

Potential sources of bias may exist in relation to the transcutaneous measurements. First of all, the skin type at the site of adhesion may affect the readings: if the skin is hardened or broken, the gases may not diffuse as well through the skin to the sensor. Sensors were carefully placed on intact normal skin to avoid this kind of bias. Another possible source of bias is possible problems in the calibration of the sensor. The calibration is performed with a stock gas at a constant partial pressure of both oxygen and carbon dioxide. However, the calibration is dependent of the state of the membrane covering the sensor. If the sensor is old or detached, the readings may be off in single recordings. However, if these kinds of bias exist in our study, it would be likely to affect each group of subjects, as equal conditions and devices were used for every patient. Finally, the ambient temperature may affect the readings of the tcCO₂ and tcO₂, since the measurement technique is susceptible to vasoconstriction and vasodilation (Healey, Fedullo et al. 1987). For this reason, the clothing of the subjects was carefully controlled during SIT sessions: all subjects were asked to wear socks and a shirt covering the site of attachment of the chest sensor. Also ambient temperature in the room was controlled automatically.

6.1.4 Skin temperature measurements

Skin temperatures were measured in our study with iButton® devices. These devices were chosen because of their reliability and accuracy in previous studies, showing the suitability of these devices in measurement and recording of human skin temperature (Hasselberg, McMahon et al. 2011, Smith, Crabtree et al. 2010, van Marken Lichtenbelt, Daanen et al. 2006). The devices are wireless and allow the recording of skin temperature for up to 96 hours with measurement interval of 120 seconds. Therefore, the devices were decided to be optimal for the requirements of this study.

Most potential sources of bias related to these measurements are related to the study design and have been mentioned before. However, also the placement of the devices on the skin may be problematic. Factors possibly affecting the readings include the skin type under the sensor, placement of the sensor closer or further from the toes and clothing covering the sensor. These factors were all addressed in planning and executing the study. The effects of skin type and clothing were addressed in the same way as in the case of transcutaneous measurements. However, during home recording, the clothing could not be controlled, although the patients were instructed to wear socks at all times. The placement of the sensor was done personally by the investigator for each patient. This way, it was ensured that the sensor was placed as close to the toes as possible in each patient.

6.2 Peripheral hypoxia and restless legs syndrome

The foremost finding of the studies presented here was the lowered oxygen partial pressure in the legs of patients suffering from RLS. This finding confirms the existence of peripheral hypoxia in RLS, suggested by molecular and histological markers in previous studies (Wählin-Larsson, Ulfberg et al. 2009, Wählin-Larsson, Kadi et al. 2007). The presence of hypoxia in RLS is also supported by the association of RLS with obesity (Gao, Schwarzschild et al. 2009) and COPD (Kaplan, Inonu et al. 2008). The hypoxia in our study was specific to the peripheral parts of the body, indicating that the problem is not in systemic blood perfusion or blood supply. The strong positive correlation of oxygen measurements with RLS severity strongly supports the hypothesis of the existence of peripheral hypoxia in RLS and indicates close involvement in RLS pathophysiology. Also the close temporal connection between the fluctuation of skin oxygen and leg movements supports the tight connection between the two phenomena.

Another major finding of the present study was the effect of pramipexole on the hemodynamics in RLS. The elevation of oxygenation and skin temperature in the periphery after the administration of pramipexole, with no effect visible on the chest, suggests redirection of blood flow from the central parts of the body to the peripheral tissues. However, surprisingly, the carbon dioxide partial pressure was not affected. Although the fact that dopamine redirects blood flow to the extremities has been demonstrated before (Eliassen, Klemp et al. 1989), we show here that pramipexole has similar effects in patients with RLS. Whether these findings can be generalized to other dopaminergics and other types of patients is

left unanswered by our data. Further studies are necessary to answer these questions.

In previous studies, signs of tissue hypoxia have been found specifically in the skeletal muscle tissues of the legs of patients with RLS (Wåhlin-Larsson, Kadi et al. 2007, Wåhlin-Larsson, Ulfberg et al. 2009). In our study, the measurements were performed on the peripheral skin tissue. This could indicate that the oxygen transport is compromised in the periphery, regardless of the tissue type. Unfortunately, no direct peripheral blood flow analyses have been performed in sufficiently large populations of untreated patients with RLS. These kinds of studies could determine in the future, if the factor causing these findings is impaired oxygen transport to the peripheral tissues or reduced total blood flow to the periphery.

There has been one previous study about blood flow in RLS. Anderson and colleagues showed in 2013 that both microvascular blood flow and skin temperature were abnormally increased in RLS patients in certain conditions (Anderson, Di Maria et al. 2013). However, they enrolled both RLS patients with and without ongoing dopaminergic medication into their study. Our skin temperature data shows that the data presented in the previous study could be confounded by the effect of the pharmacological therapy, rather than demonstrating a feature of RLS.

Contrary to the oxygen findings, the skin temperatures and partial pressures of carbon dioxide during SIT did not differ between RLS patients and healthy controls. No differences were observed in the daytime patterns of skin temperature either. These findings suggest that the blood flow to the periphery is normal in patients with RLS. The reduced oxygen partial pressure in RLS in the absence of lowered blood perfusion to the skin, measured with skin temperature as the surrogate, could be explained by at least two alternative theories.

The blood flow to the hind limbs is normal in RLS, as indicated by skin temperature measurements, but the delivery of oxygen to the periphery is compromised. The lowered partial pressure of oxygen could be explained by abnormalities in iron metabolism, reducing the blood oxygen transportation capacity to the tissues. Problems in iron metabolism have been previously suggested in the systemic circulation of patients with RLS (Earley, Ponnuru et al. 2008), but oxygen transportation capacity has not been investigated. Pramipexole would, in this case, increase the blood flow to the periphery, elevating both the skin temperature and oxygen content, as observed. Thus, pramipexole would not be fixing an abnormal baseline blood flow, but rather exaggerating the normal

blood flow to compensate the lowered capacity to transport oxygen to the periphery.

Alternatively, carbon dioxide and heat diffuse faster between the capillaries and tissue than oxygen. If that is the case, the lowering of blood perfusion of a tissue would first affect the partial pressure of oxygen and only later be seen as increased carbon dioxide and decreased tissue temperature. In RLS, a marginally lowered blood flow to the hind limbs could only be seen as low oxygen in the absence of changes in skin temperature or carbon dioxide. Pramipexole could amplify the capillary blood flow which would be seen as increased oxygen partial pressure and tissue temperature.

Our data suggests that the peripheral oxygen is linked to the pathophysiology of RLS. The question arising from this finding is, whether the observed peripheral hypoxia is a secondary marker of the RLS symptoms or their primary trigger. As a secondary phenomenon, the findings could be explained by the changes in iron metabolism. Iron changes have been extensively studied in RLS and have been shown to play a major part in RLS pathophysiology (Allen, Earley 2007). In addition to brain changes, iron metabolism is compromised in the lymphocytes of the systemic circulation (Earley, Ponnuru et al. 2008). If the oxygen transport capacity of the blood circulation was compromised as a consequence of an iron pathology, it could explain our findings.

On the other hand, the lack of oxygen in the legs could be a primary trigger of the RLS symptoms. The low oxygen partial pressure could trigger the firing of peripheral nociceptive C fibers in the legs, resulting in a sensation difficult to localize or describe. The C fibers act as metabolic sensors in the periphery, and are reactive, among other things, to hypoxia (Craig 2002). The idea of a peripheral signal from the legs as a start point of the RLS symptoms has been suggested before by the pathophysiological theory involving the A11 neural pathways (Clemens, Rye et al. 2006). According to that model, the spinal cord is lacking dopaminergic supraspinal inhibition and therefore reacts abnormally to peripheral signals. Therefore, the results presented here could be supportive to the A11 theory.

Alternatively, a new model of RLS pathophysiology, previously only introduced in conference presentations, could explain these findings. According to this hypothetical model, the RLS symptoms result from lowered oxygen levels or pH in the peripheral skeletal muscle tissues in the evening and night time, along with the decreasing sympathetic tone. This would create a C fiber activation, which is conveyed to the spinal cord and to the cortex, producing responses as leg

movement, but also as heart rate elevations. The goal of these responses would be to direct more blood flow to the compromised muscle tissues in the periphery, through increasing blood pressure and movement-induced vasodilation (Clifford, Hellsten 2004) in the tissue itself. This pathophysiological model is summarized in Figure 17.

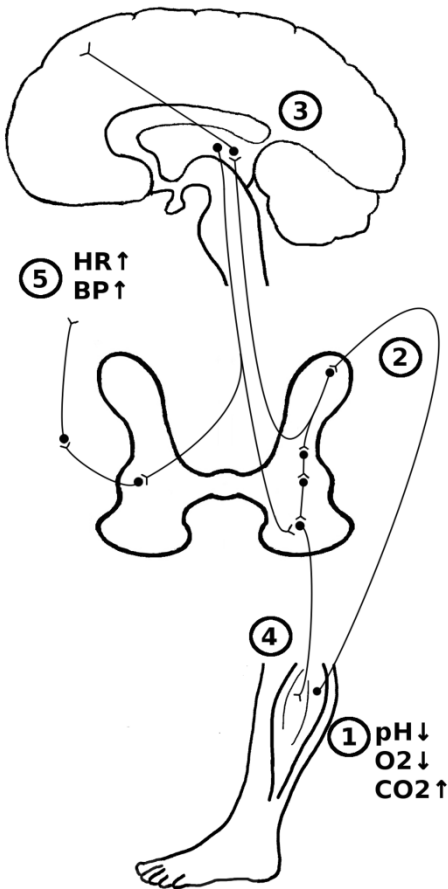


Figure 17. The suggested pathophysiological model of RLS is illustrated in simplified schematic form. The worsened metabolic conditions in the skeletal muscles of the legs, possibly due to lowered blood perfusion, trigger the activation of interoceptive C fibers (1). The signal reaches the spinal cord through the dorsal horn (2). From the spinal cord, the signal is transmitted to the thalamus and to the cerebral cortex (3). Meanwhile, a reflex arch is activated in the spinal cord through interneurons. This activates the leg muscle to a contraction (4), which can be seen as involuntary leg jerks, seen as PLM. The autonomic nervous system is activated in the process (5), producing transient elevations in heart rate (HR) and blood pressure (BP), as well as vasoconstriction on the skin.

6.3 The mechanism of periodic leg movements

This work provided two major findings in relation to PLM during sleep. Firstly, the PLM were shown to be associated with simultaneous peripheral transient vasoconstriction. This finding provides further evidence for the activation of the autonomic nervous system in relation to PLM, demonstrated previously by elevations in heart rate (Ferri, Zucconi et al. 2007) and blood pressure (Pennestri, Montplaisir et al. 2007). Secondly, our results showed that PLM may be generated without connection to the brain in a patient with complete spinal cord injury. Both of these results could advance our understanding of the mechanism of these movements.

The transient vasoconstriction, described through changes in pulse wave amplitude, was present in half of our patients suffering from RLS. We could not identify factors predicting these findings, as they were not related to RLS severity, age or gender. The discrepancy could have been, however, explained by placement of the sensor or false negative findings. We found that often when the sensor was placed in the contralateral leg compared to the movements, no vasoconstriction was observed. However, this did not explain all differences between patients. False negative findings, on the other hand, could have been caused by the frequent signal saturation or movement artefacts, hiding the vasoconstriction responses.

The effect of dopamine was another factor of interest in our study. Our findings suggest that, in addition to suppressing leg movements, dopaminergic medication also suppresses the transient events of vasoconstriction related to them. This is interesting, providing new insight into the previous findings showing that pramipexole does not affect the cortical arousals related to PLM (Manconi, Ferri et al. 2012). This suggests that the transient peripheral vasoconstriction events are directly related to the leg movements and possibly not to the cortical CAP.

Our study in spinal cord injury, on the other hand, revealed several novel aspects related to the mechanism of PLM. First of all, we showed that PLM may appear in complete absence of a neural connection to the brain. Even if the movements observed were not related to RLS, we were able to exclude the possibility of them being general muscle spasticity or myoclonus through analysis of leg movement interval and concomitant medication.

The movements observed were also temporally disconnected from cortical arousals, as well as from cardiac responses. This disconnection is in line with the pharmacological disconnection demonstrated in earlier studies (Manconi, Ferri et al. 2012). These findings are contradictory to the hypothesis suggesting that PLM originate in the dopaminergic system of the brain (Montplaisir, Lorrain et al. 1991).

Therefore, at least in this subject, the origin of the movements is somewhere below the level of lesion. Likely sites of the trigger of the movements are the spinal cord or peripheral systems.

Another important finding was that dopaminergic therapy is able to suppress PLM even in the absence of a connection to the brain. This means that in addition to the origin of the movements, also the site of action of pramipexole in the suppression of PLM is somewhere at the level of the spinal cord or periphery. Also this is in contrast to the current thinking of cerebral effects of dopaminergic medication.

The obvious limitation of this study is its nature as a case study. The findings should be investigated in the future in a larger sample of patients with complete or partial spinal cord injury. However, patients with a heavy PLM burden and complete cervical spinal cord injury may be difficult to find for larger studies. Another limitation is the lack of measurement of evoked potentials in order to confirm the complete nature of the spinal lesion. However, MRI imaging and motor and sensory classification of the injury in our subject give a fair degree of certainty of the severity of the lesion.

Although these findings have to be regarded as initial descriptive findings, they provide new insight into the mechanism behind the generation of PLM. They are in line with the previously suggested theory of spinal pacemakers, responsible for the periodicity of PLM (Manconi, Ferri et al. 2012). The spinal pacemakers could be responsible for generating movements in the absence of sufficient supraspinal inhibition. This lack of inhibition could be due to the malfunction of descending A11 neurons (Clemens, Rye et al. 2006). In this case, the administered dopamine would replace the lacking spinal dopamine from A11 neurons and suppress the movements. Alternatively, a peripheral noxious stimulus could be responsible for the generation of the movements. Pramipexole could then, as shown by our RLS studies, increase peripheral blood flow and suppress the noxious stimulus and therefore the movements. The validity of these theories needs to be addressed in future studies in order to get closer to the basic mechanism of the generation of PLM.

7 Summary and conclusions

RLS, along with the related periodic leg movements, has been generally seen as a neurological disorder generated by abnormalities in the central nervous system, although no common consensus on the mechanism of the generation of the symptoms has been found. Therefore, the findings of potential signs of hypoxia in the periphery of RLS patients have not been investigated further. Even if the body of evidence for peripheral involvement in RLS is currently not extensive, it is consistent and deserving of further analysis. The general aim of the present work was to test the hypothesis of peripheral involvement in RLS and PLM through measuring peripheral blood circulation in different situations and with different techniques.

The main conclusions of this work were the following:

1. RLS is associated with mild hypoxia, but not hypercapnia, of the peripheral cutaneous tissue during the symptomatic period. The hypoxia was specific to the peripheral parts of the body, being visible neither on the skin of the chest or in SaO₂ measurements. The hypoxia was more pronounced in patients with more severe RLS symptoms. The administration of pramipexole normalized the observed hypoxia, possibly through redirection of blood flow to the peripheral tissues.
2. Both proximal and distal skin temperatures are similar in RLS patients and healthy controls, both during the symptomatic period and during daytime. However, pramipexole increased the peripheral skin temperatures in RLS patients, supporting the finding of redirected blood flow.
3. PLM, both during wakefulness and sleep, are associated with peripheral transient vasoconstriction in a part of patients with RLS. Vasoconstriction events are suppressed with the PLM after administration of pramipexole, suggesting tight causal relationship between the movements and the vascular responses.

4. PLM may occur in patients with complete cervical spinal cord injury. In these patients, PLM may be temporally dissociated from cortical arousals, as well as from heart rate elevations. PLM may be suppressed by pramipexole even in the absence of a connection to the brain. These results challenge the theory of cerebral origin of PLM.

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Original publications

Peripheral hypoxia in restless legs syndrome (Willis-Ekbom disease)



Aaro V. Salminen, MSc
Ville Rimpilä, MSc
Olli Polo, MD, PhD

Correspondence to
Aaro V. Salminen:
aaro.salminen@unesta.fi

ABSTRACT

Objective: A case-control study to measure oxygen and carbon dioxide partial pressures in the legs in order to assess the involvement of peripheral hypoxia or hypercapnia in the pathogenesis of restless legs syndrome (RLS).

Methods: RLS severity was assessed with a standard questionnaire. Suggested immobilization tests were performed twice in 15 patients with RLS and 14 healthy controls. Patients with RLS participated in the tests with and without pramipexole medication. During the tests, peripheral oxygen and carbon dioxide partial pressures were measured noninvasively on the skin of the legs and the chest.

Results: During immobilization, the patients with RLS had lower partial pressure of oxygen in their legs (5.54 vs 7.19 kPa, $p < 0.01$) but not on the chest (8.75 vs 8.20 kPa, $p = 0.355$). More severe RLS correlated with high chest-to-foot oxygen gradient ($\rho = 0.692$, $p < 0.01$). Carbon dioxide levels did not differ between the groups. Pramipexole corrected the peripheral hypoxia toward the levels observed in the controls (from 5.54 to 6.65 kPa, $p < 0.05$).

Conclusions: Peripheral hypoxia is associated with the appearance of RLS symptoms. Strong correlation with RLS severity suggests a close pathophysiologic link between peripheral hypoxia and the symptoms of RLS. This is further supported by the simultaneous reversal of hypoxia and discomfort by dopaminergic treatment. *Neurology*® 2014;82:1856-1861

GLOSSARY

IRLSSG = International Restless Legs Syndrome Study Group; **ptco₂** = partial pressure of carbon dioxide; **pto₂** = partial pressure of oxygen; **RLS** = restless legs syndrome; **Sao₂** = arterial oxyhemoglobin saturation; **SIT** = suggested immobilization test; **tcco₂** = transcutaneous carbon dioxide; **tco₂** = transcutaneous oxygen.

Restless legs syndrome (RLS) is characterized by paresthesia typically occurring at rest. The uncomfortable feeling is almost exclusively present in the most peripheral part of the body, the legs.¹ During immobilization, the discomfort in the legs increases in a crescendo pattern² until relieved by leg movement. The symptoms are effectively relieved by dopaminergic treatment.³

The origin of RLS is currently debated. Recently, several studies have found evidence for abnormal peripheral microvasculature in patients with RLS, originally suggested by RLS pioneer Karl-Axel Ekbom in 1945.⁴ Microvascular abnormalities have also been suggested by blood flow studies,⁵ as well as by genetic studies demonstrating the possible involvement of nitric oxide.⁶ Vascular endothelial growth factor upregulation⁷ and capillary tortuosity⁸ in the legs of patients with RLS provide indirect evidence for peripheral hypoxia. However, hypoxia has not been demonstrated to be present in patients with RLS. Because hypoxic pathways may be activated by other mechanisms in normoxic conditions,⁹ direct measurement of oxygen is needed to determine whether hypoxia has a role in the activation of these pathways.

The partial pressures of oxygen and carbon dioxide (ptO₂ and ptCO₂) in tissue are affected by blood perfusion through the microvasculature, in addition to arterial oxygen supply. Therefore, we hypothesized that the suggested microvascular abnormalities would result in abnormal ptO₂ and ptCO₂ levels in patients with RLS during the symptomatic period. In this study, we used transcutaneous measurements to evaluate the oxygen and carbon dioxide levels in the peripheral tissues in patients with RLS with and without dopaminergic therapy.

Editorial, page 1850

From the University of Tampere (A.V.S., V.R., O.P.), School of Medicine; Unesta Research Centre (A.V.S., V.R., O.P.), Tampere; and Department of Respiratory Medicine (O.P.), Tampere University Hospital, Finland.

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METHODS Subjects. A total of 29 subjects were included in the study: 15 patients previously diagnosed with idiopathic RLS and currently treated with pramipexole, and 14 age- and sex-matched controls without RLS symptoms in the subject or in close family. The diagnosis of RLS was confirmed by an experienced specialist in sleep medicine and RLS (author O.P.) according to the standard diagnostic criteria.¹⁰ All subjects with significant other medical conditions were excluded. All subjects in the RLS group had plasma ferritin above 15 $\mu\text{g/L}$ in previous measurements.

Procedures. Pramipexole medication of the patients with RLS was discontinued at least 2 weeks before the start of the study. After 2 weeks off pramipexole, RLS severity was evaluated with the standard International RLS Study Group (IRLSSG) scale.¹¹ Two sessions of suggested immobilization tests (SITs) were performed in a sleep laboratory environment, 2 and 4 hours before bedtime. SIT is a standardized test to objectively assess the symptoms of RLS.¹² Multiple SITs are recommended for accurate evaluation of the symptoms.¹³ The SIT sessions lasted for 60 minutes, during which the patients were asked to rest in a bed in a semisitting position and to avoid moving their legs voluntarily. Neither sleeping nor talking, reading, or any other stimulatory activity was allowed during the tests. Two days after the first SITs, pramipexole medication was discontinued at the effective dose the patient was taking before the discontinuation (dose ranging from 0.25 to 0.50 mg). During the second evening on pramipexole, the patients returned to the study center for 2 consecutive SIT sessions. Control subjects did not take any dopaminergic medication.

During the SITs, arterial oxyhemoglobin saturation (SaO_2) was measured from the second toe of the foot. Transcutaneous carbon dioxide and oxygen (tcCO_2 and tCO_2) signals were simultaneously recorded both from the chest and from the sole of the foot with TCM4 devices paired with Severinghaus type E5280 sensors (Radiometer, Copenhagen, Denmark). Severinghaus correction and metabolic correction factors were disabled from the devices in order to measure the gas partial pressures in the tissues instead of estimating the arterial values. Chest-to-foot gradient of the oxygen and carbon dioxide levels (e.g., ptO_2 gradient = $\text{ptO}_2[\text{chest}] - \text{ptO}_2[\text{foot}]$) were calculated to estimate the distribution of the gases between the central and peripheral parts of the body. During the SIT, subjects

were prompted every 5 minutes to record the current level of leg discomfort on a scale ranging from no discomfort (value 0) to maximal discomfort (value 10). At each time point, the values of tcCO_2 , tCO_2 , and SaO_2 measurements were recorded. A mean value of these parameters in the 2 SITs was used in the analyses.

Statistical methods. We tested 3 primary hypotheses for both the oxygen and carbon dioxide measurements: (1) peripheral partial pressures are altered in patients with RLS compared with controls, (2) the measured partial pressures show correlation with IRLSSG scale score of RLS severity, and (3) peripheral levels are modified by pramipexole treatment in patients with RLS. Carbon dioxide and oxygen values between the patients and individually matched controls were compared at each time point during the SIT with 2-tailed Mann-Whitney test. Measurements in patients with RLS with and without medication were compared with 2-tailed Wilcoxon signed-rank test. Correlations were evaluated with Spearman rank correlation coefficient.

Standard protocol approvals, registrations, and patient consents. The study was approved by the local ethical committee (Pirkanmaa Hospital District, Tampere, Finland). All patients signed informed consent before any study procedures.

RESULTS The patient and control subject groups were matched for age and sex. The mean ages were 57.1 and 56.6 years, respectively. Seven of 15 patients and 6 of 14 controls were women. All patients had been previously diagnosed with idiopathic RLS. The mean RLS severity after the discontinuation of pramipexole medication was 23.7 in the RLS patient group. One subject in each subject group had to be excluded because of insufficient quality of the transcutaneous recording. Patients with RLS who did not have significant RLS symptoms (IRLSSG scale severity <15 , $n = 3$) 2 weeks after discontinuation of pramipexole were excluded from the analyses comparing patients with RLS and controls, but not from the analysis of correlation between RLS severity and measured values.

The mean values of subjective discomfort, ptO_2 , ptCO_2 , and SaO_2 levels during SIT in the different patient groups are presented in the table. The levels of ptO_2 and ptCO_2 on the chest did not differ between the patient and control groups. In contrast, patients with RLS had lower oxygen levels in their legs during the SIT compared with controls. Also the chest-to-foot ptO_2 gradient differed significantly between the 2 groups. There was no difference between the groups in the measurements of carbon dioxide or SaO_2 .

The ptO_2 gradient had a strong, statistically significant correlation with RLS severity: the more severe RLS the patient had, the greater the difference in ptO_2 was between the chest and the periphery (Spearman correlation $\rho = 0.692$, $p < 0.01$). Moreover, the more serious cases of RLS also had higher levels of absolute ptCO_2 in their legs ($\rho = 0.624$, $p < 0.05$). Scatter plots of these parameters, plotted against RLS severity, are displayed in figure 1. Other measurements of oxygen and carbon dioxide did not correlate significantly with RLS severity in our patient population (foot ptO_2 : $\rho = -0.404$, $p = 0.152$; and ptCO_2 gradient: $\rho = -0.463$, $p = 0.095$).

Table Mean values of discomfort and partial pressures of oxygen and carbon dioxide during SIT

	RLS	Control	<i>p</i>	RLS (PPX)	<i>p</i>
Discomfort	4.2	0.1 ^a	<0.001	1.6 ^a	<0.001
Foot ptO_2 , kPa	5.54	7.19 ^b	<0.01	6.65 ^c	<0.05
Chest ptO_2 , kPa	8.75	8.20	0.355	7.86	0.156
ptO_2 gradient, kPa	3.22	0.93 ^b	<0.01	1.21 ^c	<0.05
Foot ptCO_2 , kPa	6.82	6.92	0.955	7.24	0.173
Chest ptCO_2 , kPa	6.69	6.80	0.710	7.16	0.117
ptCO_2 gradient, kPa	-0.12	-0.11	0.691	-0.04	0.594
SaO_2	97.7%	97.7%	0.955	97.5%	0.477

Abbreviations: PPX = pramipexole; RLS = restless legs syndrome; SaO_2 = arterial oxyhemoglobin saturation; SIT = suggested immobilization test.

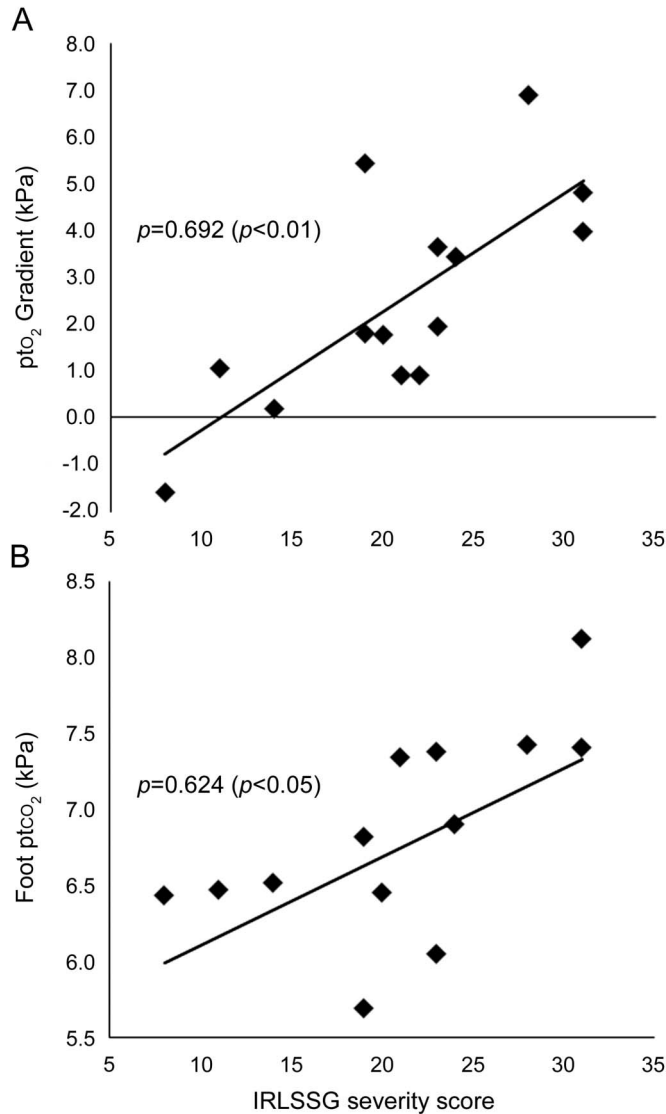
RLS and control values represent the mean. The subjective discomfort was assessed with a scale ranging from 0 to 10. The measurements were performed in patients with RLS on 2 occasions (on and off PPX). In healthy controls, the values are means of 4 SIT sessions measured during 2 evenings. The *p* values are shown in comparison to drug-free patients with RLS.

^a*p* < 0.001.

^b*p* < 0.01.

^c*p* < 0.05.

Figure 1 Linear regression of the chest-to-foot ptO_2 gradient and foot $ptCO_2$ vs restless legs syndrome severity



Values of the chest-to-foot gradient of partial pressure of oxygen (ptO_2 , A) and foot partial pressure of carbon dioxide ($ptCO_2$, B) plotted against International Restless Legs Syndrome Study Group (IRLSSG) severity scale scores.

The recontinuation of pramipexole did not have an effect on ptO_2 on the chest but increased the mean foot ptO_2 and therefore decreased ptO_2 gradient during SIT (table). Carbon dioxide levels or distribution did not change with treatment.

Figure 2 displays the evolution of oxygen and carbon dioxide measurements, as well as the subjective discomfort, during the SIT in each patient population. Although the effects of pramipexole on foot $ptCO_2$ were not significant when comparing mean levels during SIT, the effect was significant at several individual time points during the test, especially toward the end of the immobilization. The effect of pramipexole on foot ptO_2 was not statistically significant at some time points.

In the most severe of our RLS cases ($n = 3$, IRLSSG scale severity ranging from 28 to 31), the patterns of leg

movements and ptO_2 measured on the foot were closely connected during SIT (example in figure 3): the ptO_2 was decreasing spontaneously when legs were still and relaxed. When a movement occurred, the oxygen level increased momentarily and started to decrease again after relaxation. This pattern was not observed in cases of mild to moderate RLS.

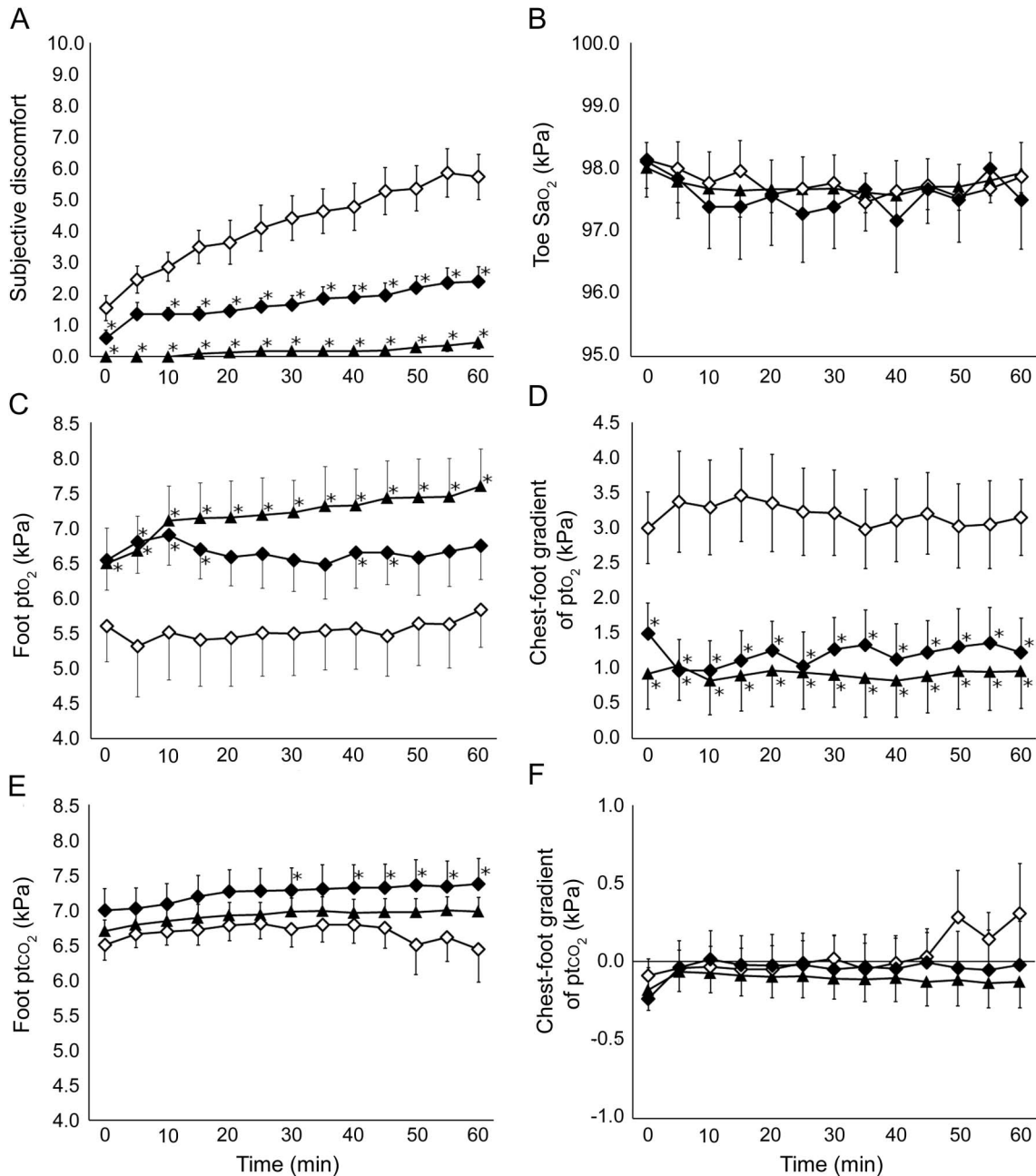
DISCUSSION Most previous studies on RLS have focused on the CNS. Our data add to the emerging body of evidence supporting the hypothesis that peripheral phenomena also contribute to the pathophysiology of RLS. The most important new finding of our study is the demonstration of peripheral hypoxia in patients with RLS and its close relation to RLS severity, supporting the hypothesis of microvascular abnormalities in RLS. In addition, the restoration of peripheral oxygenation with pramipexole, the first-line therapy for RLS,¹⁴ is in agreement with the thinking that peripheral hypoxia may not only be involved in symptom generation, but its correction may also mediate some of the treatment effect. These findings are consistent with our hypothesis that peripheral systems are involved in RLS pathophysiology.

It is unlikely that the lowered ptO_2 , observed in patients with RLS, could have been caused by changes in partial pressure of arterial oxygen, because no changes were observed in the peripheral SAO_2 . To control for potential bias from individual systemic differences in skin oxygenation, ptO_2 and $ptCO_2$ were measured simultaneously on the skin of the chest. The chest measurements showed no difference between the 2 patient groups. These findings demonstrate that in RLS, lowered oxygen levels are specific to the peripheral parts of the body and are not a systemic phenomenon.

There was a strong positive correlation between the severity of RLS symptoms when off pramipexole and the chest-to-foot gradient of tissue oxygen. This suggests that peripheral hypoxia is not only a circumstantial finding during the moment of immobilization but also a feature closely related to the degree of subjective RLS complaint. The finding supports the involvement of hypoxia in RLS pathophysiology either as a primary trigger or a closely related secondary phenomenon.

The low ptO_2 in the extremities, characteristic of RLS, was corrected with a standard dose of pramipexole. The treatment simultaneously resolved the RLS discomfort. The mechanism of action of dopamine agonists in RLS is generally thought to be in the CNS,¹⁵ although the exact site of action has not yet been identified. Dopamine receptors are present in all parts of the body. In systemic arteries,¹⁶ dopamine is a potent arterial vasodilator,¹⁷ and it enhances blood flow in subcutaneous fat tissue and peripheral skeletal muscles.¹⁸ The redirection of blood flow to the periphery could potentially explain our findings. The fact that pramipexole simultaneously

Figure 2 Development of discomfort, and oxygen and carbon dioxide during SIT



Evolution of subjective discomfort (A), arterial oxyhemoglobin saturation (B), and the foot partial pressures and chest-to-foot gradients of partial pressures of oxygen (ptO₂, C and D) and carbon dioxide (ptCO₂, E and F) during SIT. Subject groups are patients with RLS without therapy (◊), patients with RLS receiving pramipexole (◆), and healthy controls (▲). *p < 0.05 compared with the RLS group without therapy. RLS = restless legs syndrome; Sao₂ = arterial oxyhemoglobin saturation; SIT = suggested immobilization test.

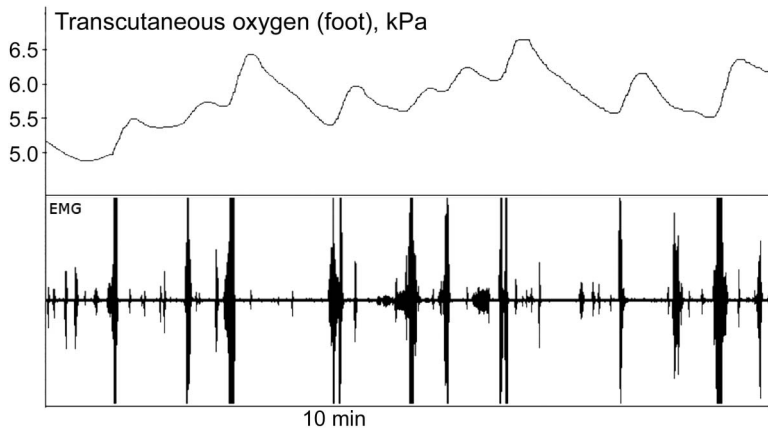
corrects both subjective discomfort of RLS and peripheral oxygenation, could support the existence of a pathophysiologic link between the 2 phenomena.

At baseline, the ptCO₂ levels did not differ significantly between the 2 groups. However, there was a positive correlation between the RLS severity and leg ptCO₂ during SIT: higher ptCO₂ levels were associated with more severe RLS symptoms. We were expecting to find a difference in the carbon dioxide levels between the groups, reflecting compromised blood flow to the lower

limbs. The finding could mean that instead of reduced blood flow, other mechanisms, such as impaired oxygen delivery to peripheral tissues, should also be considered. Pramipexole did not have an effect on the mean ptCO₂ in the legs but the effect was significant at single time points toward the end of the SIT. A larger population would be needed to assess this effect in more detail.

In the most severe cases of RLS, the pattern of the fluctuation of tissue oxygen and the appearance of leg movements closely resembled the crescendo pattern of

Figure 3 Movement and ptO_2 patterns during SIT



An example of a SIT test recording of a patient with severe restless legs syndrome (IRLSSG scale severity = 31), in whom the ptO_2 decreased before and increased after movements. The patient was reporting maximum discomfort (10/10) at the time of the recording. IRLSSG = International Restless Legs Syndrome Study Group; SIT = suggested immobilization test.

the RLS symptoms. The similarity between the pattern of the subjective symptoms and objective measurements provides further evidence for a close connection between the peripheral ptO_2 levels and RLS discomfort.

These findings imply that impaired delivery of oxygen to the periphery has a role in the pathogenesis of RLS. Peripheral hypoxia could be a primary trigger of RLS, irritating peripheral afferent nerves. Afferent signals from muscle nerves have been suggested to be involved in the generation of RLS symptoms by the A11 pathophysiologic model.¹⁹ Irritation of these nerve fibers in the periphery could trigger the uncomfortable sensations through the nociceptive system. Alternatively, hypoxia could be a secondary phenomenon resulting from deficiencies in iron metabolism, often associated with RLS.²⁰ Impaired iron metabolism has been demonstrated not only in the basal ganglia of the brain but also in blood lymphocytes.²¹ Impaired iron metabolism in the peripheral skin tissue could compromise the oxygen uptake to the tissue and explain the current findings. However, more data are needed to forward this hypothesis.

Peripheral hypoxia could explain some of the earlier findings in RLS. It could provide an explanation for the upregulation of vascular endothelial growth factor in the skeletal muscles of the legs in RLS,⁷ as well as the capillary tortuosity in the skeletal muscles of the same tissues.⁸ Both of these findings are signs of tissue hypoxia. RLS has also been associated with activation of a hypoxia-inducible protein (HIF-1) in substantia nigra,²² but this hypoxic marker has not been assessed in the peripheral tissues. A central role of hypoxia in the pathogenesis of RLS could also explain the high prevalence of RLS symptoms in disorders associated with hypoxia, such as chronic obstructive pulmonary disease²³ or obstructive sleep apnea.²⁴ In addition, the possible beneficial use of different compression devices in RLS²⁵

could be explained by the involvement of peripheral oxygen in RLS pathogenesis.

The simultaneous effect of pramipexole on both the peripheral oxygen levels and RLS symptoms could further support a primary role of hypoxia in RLS pathophysiology. If hypoxia was a primary trigger of RLS symptoms, the correction of peripheral oxygen levels by dopaminergic therapy would efficiently suppress the symptoms. A recent study showed that the cerebral effects of dopaminergic agents are not necessary for their therapeutic effect on periodic leg movements in a patient with complete spinal cord lesion.²⁶ The data presented here raise the hypothesis that the same could be true in RLS.

In this study, we used the transcutaneous method to assess ptO_2 and $ptCO_2$. Pulse oximetry is the conventional technique to assess oxygenation, measuring SaO_2 . The ptO_2 and $ptCO_2$ levels are affected by SaO_2 , but also by blood perfusion, and are therefore sensitive to vasoconstriction. Transcutaneous measurements may be used to noninvasively estimate the levels of ptO_2 and $ptCO_2$ in skin tissue.²⁷ Transcutaneous O_2 is used in the diagnosis of peripheral vascular insufficiency,²⁸ and agrees well with MRI measurements of oxygen in certain patient groups.²⁹ Transcutaneous CO_2 is used to evaluate limb ischemia in surgery.³⁰ Therefore, we believe that tCO_2 and tCO_2 are the most appropriate methods to continuously monitor ptO_2 and $ptCO_2$ in skin.

There are several limitations to the interpretation of our results. Although the tCO_2 method has been shown to correlate with MRI measurements of the oxygen content in underlying muscle tissue,²⁹ the method remains insufficiently validated in different patient populations. The signal could be affected by various other factors, including vasoconstriction.³¹ Interpretation of the signal is therefore difficult and it may not fully translate to ptO_2 of the underlying tissues. Another limitation is that this is the first study to explore the connection between RLS and peripheral oxygen levels, and the results require confirmation in future studies, perhaps in larger patient populations.

Taken together, our data provide a set of findings that increases the evidence for the involvement of peripheral factors in RLS pathophysiology. The findings are consistent in supporting the association of RLS with peripheral hypoxia and provide an explanation for the previously described activation of hypoxic pathways in RLS. Moreover, our data provide a new potential mechanism of action for dopaminergic therapy in suppressing RLS.

AUTHOR CONTRIBUTIONS

Aaro V. Salminen: drafting or revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data. Ville Rimpilä: drafting or revising the manuscript, study concept or design. Olli Polo: drafting or revising the manuscript, study concept or design, analysis or interpretation of data, acquisition of data, study supervision or coordination.

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DISCLOSURE

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Pramipexole Alters Thermoregulation in Restless Legs Syndrome

Aaro V. Salminen, M.Sc.^{1,2}; Ville Rimpilä, M.Sc.^{1,2}; Olli Polo, M.D., Ph.D.^{1,2,3}

¹University of Tampere, School of Medicine, Tampere, Finland; ²Unesta Research Centre, Tampere, Finland;

³Department of Respiratory Medicine, Tampere University Hospital, Tampere, Finland

Study Objectives: Previous studies have associated restless legs syndrome (RLS) with peripheral hypoxia and impaired thermoregulation in the lower extremities. We performed long-term monitoring of skin temperatures in order to investigate whether these findings could be explained by reduced blood flow to the peripheral tissues.

Methods: 96-hour continuous measurements of skin temperature were performed both in the distal and proximal parts of the body of 15 patients with RLS and 14 healthy controls. During the recording, the patients participated in suggested immobilization tests both with and without pramipexole medication.

Results: We found no baseline differences in distal or proximal skin temperature between patients and controls in daytime or during immobilization. However, pramipexole significantly

increased distal skin temperature in the patient group during immobilization (31.1°C vs. 32.9°C, $p < 0.05$). Daytime temperatures were not affected by therapy or disease status.

Conclusions: The data suggest that patients with RLS and healthy controls have similar blood flow to the peripheral skin tissue. Pramipexole, however, alters thermoregulation and the previous studies might have been biased by medication. Dopaminergic medication is a major confounding factor when assessing peripheral phenomena in RLS and should be controlled for in the future studies.

Keywords: restless legs syndrome, skin temperature, thermoregulation, pramipexole

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Restless legs syndrome (RLS), recently known as Willis-Ekbom disease, is associated with discomfort precipitated by immobility in the evening hours.¹ Traditionally, RLS is believed to be of central origin, but recently RLS has also been associated with several findings suggesting peripheral abnormalities. Most importantly, a recent study suggested the appearance of peripheral hypoxia during immobilization in patients with RLS.² In addition, the skeletal muscles of the legs of RLS patients have been shown to have higher capillary tortuosity³ and up-regulated vascular endothelial growth factor (VEGF).⁴ These findings imply that these patients suffer from peripheral hypoxia. The hypoxia could result from insufficient blood flow to the legs either during daytime or only during immobility. To assess this hypothesis, continuous blood flow measures are warranted in RLS patients.

Continuous measurement of skin blood flow may be achieved by measuring changes in skin temperature. Skin temperatures are commonly measured both distally and proximally, and a distal-to-proximal gradient (DPG) is calculated to assess the blood flow to the distal parts of the body.⁵ Distal skin temperature rises at sleep onset while the DPG of skin temperature shifts closer to zero, coinciding with the increase in melatonin secretion.⁶ These data show that DPG is a selective tool for continuously assessing the thermoregulatory blood flow.

Body temperature could be an important factor in RLS. Patients with RLS often complain of feelings of hot or cold temperature in the legs. Idiopathic RLS is associated with impaired perception of temperature resulting from impaired

BRIEF SUMMARY

Current Knowledge/Study Rationale: Recent studies have shown impaired thermoregulation in medicated patients with restless legs syndrome (RLS). We investigated the effect of pramipexole on thermoregulation in RLS.

Study Impact: Our results demonstrate that pramipexole modifies the thermoregulation in patients with RLS at the time when symptoms occur. Dopaminergic therapy should be well controlled for in future studies assessing peripheral blood flow in RLS.

somatosensory processing of the stimulus.⁷ Abnormal pain responses to heat stimulus have also been reported in RLS.⁸ Thermal hypoesthesia, on the other hand, is associated with secondary but not idiopathic RLS.⁹ Recently, it was demonstrated that RLS could be associated with microvascular changes, manifesting as abnormal skin temperatures,¹⁰ possibly partly explaining the abnormal thermal sensations. In this study, we wanted to confirm this finding in a 96-hour continuous skin temperature measurement and assess the effect of dopaminergic medication to skin temperatures in RLS.

METHODS

Fifteen patients suffering from RLS and fourteen age- and sex-matched controls with no RLS symptoms were recruited to the study. Only idiopathic RLS patients with current pramipexole medication and no significant other medical conditions were included. All RLS patients had previously measured

Table 1—Mean values of skin temperatures during SIT.

	RLS	Control		RLS (PPX)	
	Mean	Mean	p	Mean	p
Discomfort during SIT	4.2	0.1***	< 0.001	1.6***	< 0.001
Foot skin temperature during SIT (C)	31.1	31.1	0.991	32.9*	< 0.05
Skin temperature gradient during SIT (C)	3.1	3.0	0.916	1.3*	< 0.05

Subjective discomfort (scale 0-10) and skin temperature measurements during SIT are displayed in drug-free patients with RLS, healthy control subjects, and RLS patients with pramipexole (PPX) medication. The p values are displayed in comparison to RLS patients without treatment.

plasma ferritin > 15 µg/L. Control subjects with known RLS in immediate family were excluded.

RLS patients discontinued their pramipexole medication ≥ 2 weeks before starting the measurements. RLS severity, measured with standard IRLSSG scale,¹¹ and sleepiness (Epworth sleepiness scale, ESS) were evaluated 2 weeks after the discontinuation of the medication. The study protocol lasted for 4 full days (96 h) starting in the evening of day one. Pramipexole medication was re-continued on day 3 of the study, at the dose previously found efficient for suppressing RLS symptoms (ranging from 0.25 to 0.5 µg). Control subjects went through the same protocol, with the exception of not having to take any dopaminergic medication.

Skin temperatures were measured continuously for the duration of 4 days with model DS1922L iButtons (Maxim Integrated, San Jose, CA, U.S.). These devices have been validated for measurement of human skin temperature and have accuracy of 0.09°C and precision of 0.05°C.¹² Skin temperature was recorded with an interval of 120 sec from the instep of both feet and parasternally from the chest. Mean of the 2 feet was used to evaluate distal skin temperature. DPG of the skin temperatures ($DPG = T(\text{foot}) - T(\text{chest})$) was calculated.

All subjects went through 4 suggested immobilization tests (SIT). They were performed in the first and fourth evening of the study, 2 and 4 h before regular bedtime. SIT is a standardized test to objectively assess RLS symptoms. The patients are asked to stay still in a semi-sitting position for 60 min.¹³ Every 5 min during the test, subjects are asked to report the subjective level of leg discomfort on a scale from 0 (no discomfort) to 10 (maximal discomfort). The momentary values of skin temperature were recorded with the same interval. Any distracting activity, such as reading or watching TV, was not allowed during the tests.

Daytime skin temperatures were analyzed as mean values in periods of 1 h. Instead of time of day, self-reported time of awakening in the morning was used as a marker to fix individual recordings to a timeframe for comparison. The analysis spanned from 2 h before awakening in the morning until 10 h after awakening. In RLS patient group, mean values for each time block was calculated separately from 2 days with and 2 days without pramipexole medication. For control patients, an average of 4 days was used.

Skin temperatures both during the SIT and during daytime were compared between the subject groups. Skin temperatures between patients and controls were compared at each time point with 2-tailed Mann-Whitney test. Measurements in RLS patients with and without medication were compared

with 2-tailed Wilcoxon signed-rank test. Correlations with RLS severity were evaluated with Spearman rank correlation coefficient. All RLS patients were included in the analysis of correlation.

The study was approved by the local ethical committee (Tampere, Finland). All patients signed informed consent before participation to the study.

RESULTS

The 2 patient groups were matched for gender and age. The patient group included 7 and the control group 6 female subjects. The mean ages in the patient and control groups were 57.1 and 56.6 years, respectively. All patients had a previous diagnosis of idiopathic RLS with plasma ferritin values in the normal range. The mean severity of RLS (IRLSSG scale) without pramipexole medication was 23.7 in the RLS patient group. No difference was found in sleepiness between RLS patients and controls (ESS score 6.1 vs. 4.3, $p = 0.275$, respectively). RLS patients who did not suffer from RLS symptoms 2 weeks after discontinuation of pramipexole (IRLSSG severity < 15, $n = 3$) were excluded from the analyses.

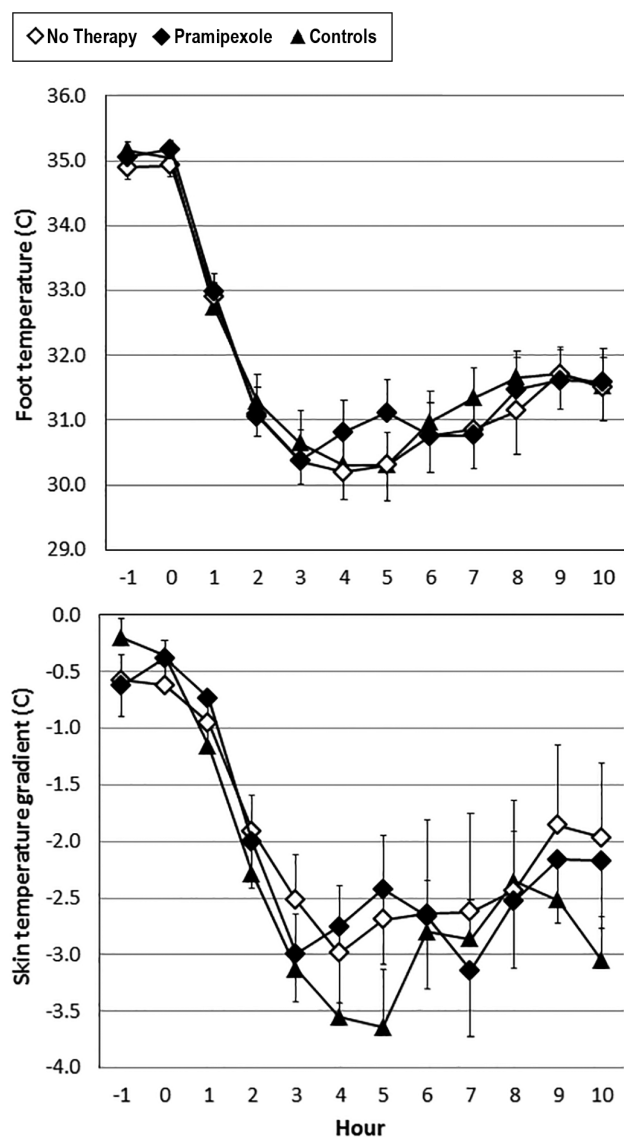
The continuous skin temperature measurements showed higher distal skin temperatures during the night, close to the level of normal core body temperature (**Figure 1**). During the day the average temperatures dropped by 4-5°C. Consequently, the DPG was close to zero during the night and dropped during daytime. No statistically significant differences were observed between RLS patients and healthy controls. Pramipexole, taken the previous evening, did not have an effect on the skin temperature during the next day.

During SIT, the RLS patients suffered from greater discomfort than the controls (**Table 1**). The skin temperatures or DPG did not differ between the 2 subject groups (**Figure 2**). Continuation of pramipexole resolved the discomfort almost entirely in the RLS patient group. Pramipexole also increased the skin temperature in the extremities but not on the chest. This can be seen as a rise in both the distal skin temperature and DPG (**Table 1**). Average skin temperature values did not correlate with RLS severity (Foot temperature; $\rho = -0.110$, DPG; $\rho = -0.221$).

DISCUSSION

The original aim of this study was to confirm the previous findings, suggesting impaired microcirculation in the extremities of patients with RLS.^{2,4} Our 96-hour skin temperature

Figure 1—The skin temperatures and skin temperature gradients are displayed as averages in each group.

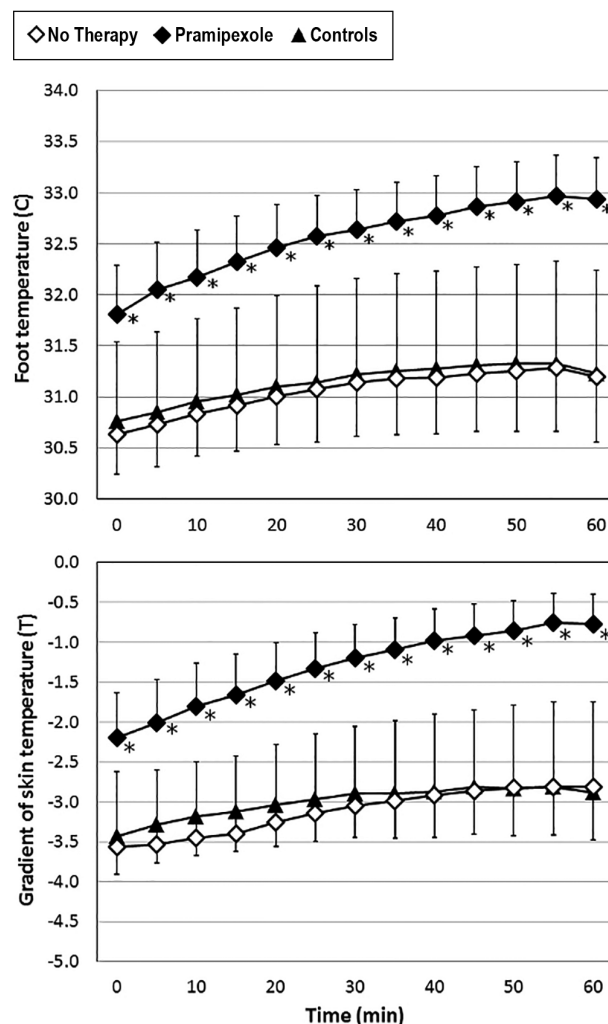


The values are average values during a period of one hour. X-axis shows the hour, zero being self-reported awakening. Subject groups are RLS patients without therapy (open diamond), RLS patients with pramipexole (closed diamond) and healthy controls (closed triangle). All differences between subject groups are nonsignificant.

measurements, however, do not support this hypothesis. Skin temperatures remained similar in the patients and controls during daytime and during the provocation of symptoms by SIT. The skin temperatures were, however, increased in RLS patients after administration of pramipexole medication. Therefore, our data suggest that increased distal skin temperatures are associated with dopaminergic treatment rather than RLS.

Skin temperature gradient has been shown to correlate with skin blood flow.⁶ If the variable is agreed to mirror skin blood flow, our data suggest no difference in blood flow to the skin between RLS patients and healthy controls. On the other hand, dopaminergic medication seems to enhance the blood flow in the peripheral skin tissue. This is supported by previous

Figure 2—The foot skin temperature and the chest-to-foot gradient of skin temperature at each time point during suggested immobilization.



Subject groups are RLS patients without therapy (open diamond), RLS patients with pramipexole treatment (closed diamond) and healthy controls (closed triangle). * $p < 0.05$ compared to RLS group without therapy.

findings suggesting that dopamine redirects blood flow from the central parts of the body to the peripheral muscles and skin in humans.¹⁴ In rats, a D1 and D2 receptor agonist apomorphine reduces the core body temperature,¹⁵ further supporting the effect of dopamine on thermoregulation.

Despite the normal baseline blood flow to the peripheral skin tissue demonstrated here, our previous results demonstrated hypoxia specific to the peripheral tissues in RLS patients.² Signs of hypoxia have also been shown in the peripheral skeletal muscle of patients with RLS.⁴ This paradox could be explained by reduced oxygen transportation capacity to the periphery. Iron is an important factor in oxygen transport, and therefore could be the underlying reason. Even if our patients had normal serum ferritin levels, changes in iron metabolism have been demonstrated in RLS patients without iron deficiency.¹⁶ Alternatively, the hypoxia findings could be related to the symptoms themselves and not to a compromised oxygen transport.

In a recent study, Anderson et al. showed impaired microcirculation in RLS, as demonstrated by laser-Doppler flowmetry and skin temperature measurements.¹⁰ However, they enrolled patients with concomitant pramipexole medication. Our data show that the results they obtained are likely to be the effect of pramipexole and not a feature of RLS. Pramipexole increases skin temperature in the legs of RLS patients, while simultaneously resolving the RLS symptoms. This effect of dopaminergic medication on skin temperature is likely to be a result of increased blood flow to the legs and therefore affect also the blood flow measurements by laser-Doppler flowmetry.

The distal skin temperature values correlate with subjective vigilance, as well as with its neurological markers.^{17,18} This together with our data could suggest that RLS patients are not less vigilant during the daytime or evening hours than healthy controls, despite the sleep loss. This is supported by similar level of sleepiness in the two groups in our study. Previous findings have shown similar results, some even finding higher daytime vigilance in RLS patients than controls.¹⁹ After treatment with pramipexole, the patients showed higher distal skin temperatures, possibly indicating reduced vigilance. Indeed, increased daytime somnolence after pramipexole treatment has been demonstrated in Parkinson disease,²⁰ and sleepiness has also been listed as a common side effect of pramipexole in RLS.²¹

The daytime pattern observed in our study is similar to those reported by previous studies. The pattern of distal skin temperature reaching values of the normal core body temperature during sleep, probably as a consequence of skin vasodilation, has been demonstrated previously.²² Lesser diurnal variation can be seen in skin temperatures on the chest, resulting in a shift in DPG upon awakening. This pattern can also be seen in our data. In our study, both RLS patients and controls, regardless of the status of medication, showed a daytime skin temperature pattern fully comparable to each other and that observed in previous studies in healthy subjects.

RLS patients often report feelings of burning in their legs²³ but have normal distal skin temperatures when not on medication, as shown by our data. This implies that the sensations are likely to be explained by decreased thermal pain thresholds or impaired somatosensory processing of the pain signal. Impaired temperature perception has been demonstrated in up to 72% of patients with idiopathic RLS.⁷ Another study shows increased temporal summation of heat pain in RLS patients compared to healthy controls.⁸ However, thresholds for thermal perception are not altered in idiopathic RLS.⁹ Taken together, these data suggest impaired central sensory processing of thermal stimuli in patients with idiopathic RLS, explaining the thermal sensations in the absence of altered skin temperatures.

Our data demonstrate that administration of pramipexole raises the distal skin temperature in patients with RLS. The effect is not visible in the morning hours, as indicated by the longtime measurement of skin temperature (**Figure 1**), possibly due to the shorter half-life of pramipexole in the body. It remains to be determined if this can be generalized to other dopaminergic therapies or to other types of subjects, including healthy controls. However, previous studies have demonstrated the redirection of blood flow from the central parts of the body to the periphery after dopamine infusion,¹⁴ suggesting that the effect could be in common to other dopaminergic therapies, as

well as subjects without RLS. Further studies are warranted to confirm the effect of other dopaminergics.

There are limitations to the methods used in our study. The continuous skin temperature measurements were performed at subjects' homes, where the ambient temperature could not be controlled. Another potential source of bias is the placement and number of iButton devices used. In many other studies, the proximal skin temperature is measured with several devices and average values are used. When only one device is used, the site is more likely to be exposed to the ambient temperature. To reduce bias from these sources in daytime recordings, the skin temperatures of patients and matching controls were always recorded simultaneously, in order to avoid bias from the outside weather and ambient temperature. During SIT, the ambient temperature and the clothing of the subjects were controlled.

In conclusion, our data demonstrates normal blood flow to the legs in RLS, despite peripheral hypoxia shown in previous studies. In addition, we show that the previously reported differences in skin temperature and peripheral blood flow could be severely biased by dopaminergic medication. In the future, RLS studies focusing on the peripheral aspects of the disease should be performed in medication-free patients to avoid bias. The absence of differences in distal skin temperatures between RLS patients and controls suggests normal peripheral blood flow in patients with RLS.

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Address correspondence to: Aaro V. Salminen, M.Sc., Unesta Research Centre, Finn-Medi 6, Biokatu 14, 33520 Tampere, Finland; Tel: +358 50 345 8970; Fax: +358 3 261 7778; E-mail: aaro.salminen@unesta.fi

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Periodic Leg Movements are Associated with Peripheral Vasoconstriction during Sleep

Authors:

Aaro V. Salminen, MSc^{1,2*}, Jussi Nupponen, BSc^{2,3}, Ville Rimpilä, MSc^{1,2}, Olli Polo, MD, PhD^{2,4}

Affiliations:

- 1) University of Tampere, School of Medicine, Kalevantie 4, 33014 Tampere, Finland
- 2) Unesta Research Centre, Finn-Medi 6, Biokatu 14, 33520 Tampere, Finland
- 3) The Department of Pervasive Computing, Tampere University of Technology, Korkeakoulunkatu 10, 33720 Tampere
- 4) Department of Respiratory Medicine, Tampere University Hospital, Teiskontie 35, 33520 Tampere, Finland

***Corresponding author:** Aaro V. Salminen, MSc, Unesta Research Centre, Finn-Medi 6, Biokatu 14, 33520 Tampere, Finland, tel: +358 50 345 8970, fax: +358 3 261 7778, email: aaro.salminen@unesta.fi

Running head: PLM and peripheral vasoconstriction

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SUMMARY

Periodic leg movements (PLM) during sleep are temporally associated with elevations in heart rate and blood pressure, indicating sympathetic activation. In this study, we evaluate if the sympathetic activation can also be seen in peripheral skin blood flow, through measurement of toe pulse-wave amplitude (PWA). In this study, ten patients with restless legs syndrome were recruited to the study. They went through a polysomnography with toe photoplethysmogram recording after discontinuation of pramipexole medication. The sleep study was repeated after the re-continuation of pramipexole. Transient changes in PWA related to the PLM were assessed. We found that half of the patients had transient dips in PWA following the start of the leg movements. The other half of the patients did not show similar dips. When present, both leg movements and PWA dips were suppressed by pramipexole. The results suggest that PLM are associated with transient peripheral vasoconstriction in some patients with RLS. The vasoconstriction is in tight temporal causal relationship with the leg movements.

Keywords: Periodic leg movements, vasoconstriction, pulse wave amplitude

INTRODUCTION

Periodic leg movements (PLM) are a common finding in polygraphic sleep studies. Most patients with restless legs syndrome (RLS) present with PLM during sleep (Montplaisir, et al. 1997), but the finding is not specific to RLS. Both RLS and PLM are efficiently suppressed by dopaminergic medication, such as pramipexole (Manconi, et al. 2007). PLM may be associated with elevated risk of cardiovascular disease (Koo, et al. 2011), but the mechanism of this association has been a subject of recent debate.

A potential mechanism for this connection may be provided by transient sympathetic activations. Sympathetic activation during sleep may be detected with several methods. The most common parameters are different heart rate variability (HRV) derivatives, which can be computed from ECG recordings and have been extensively used also in sleep recordings (Stein and Pu 2012). That method, however, requires a long steady state, which makes the usage difficult in many cases. Blood pressure may also be monitored continuously during sleep recordings to assess sympathetic activation (Bao, et al. 2002). Continuous finger plethysmogram recordings may be used to monitor the autonomic influence to skin blood flow (Grote, et al. 2003).

Sympathetic activation during PLM has been shown in previous studies. PLM are associated with transient increases in heart rate (Ferrillo, et al. 2004; Lavoie, et al. 2004) as well as in blood pressure (Pennestri, et al. 2007). According to these studies, heart rate elevations may start already before the start of the leg movement. In addition, PLM are associated with cyclic alternating pattern (CAP) in simultaneous EEG recordings (Parrino, et al. 1996), which are also considered to indicate sympathetic activation (Ferini-Strambi, et al. 2000). However, treatment with pramipexole dissociates PLM from the EEG arousals (Manconi, et al. 2012). PLM have been previously

associated with peripheral vasoconstriction in some patients (Ware, et al. 1988), but the relation has not been analyzed in detail. In this study we investigated whether the transient sympathetic activation, associated with PLM, can be seen as peripheral vasoconstriction during sleep and wakefulness.

METHODS

Ten patients with idiopathic RLS, who were currently on pramipexole medication, were included in the study. Other significant medical conditions and sleep disorders were excluded in an interview by an experienced physician. The patients discontinued their pramipexole medication at least two weeks before starting the measurements. RLS severity, measured with standard IRLSSG scale (Walters, et al. 2003) was evaluated two weeks after the discontinuation of the medication.

The patients underwent a full night polygraphic sleep recording (PSG) on the first night of the study. The PSG was carried out in sleep laboratory environment. The patients were free of any RLS medication during the first recording. The PSG setup included the recording of electroencephalogram (EEG, 200 Hz), electrooculogram (EOG, 200 Hz), submental electromyogram (EMG, 200 Hz), bilateral tibial anterior EMG (200 Hz), electrocardiogram (ECG, 2000 Hz) and pulse oxymeter on the second toe of the right foot recording the arterial oxygen hemoglobin saturation (1 Hz) and the photoplethysmogram signal (100 Hz). The photoplethysmogram signal was used to determine the pulse-wave amplitude (PWA). After the first night, pramipexole treatment was re-continued. On the second night on pramipexole treatment, the PSG was repeated with similar setup.

Sleep staging was performed in 30-second epochs according to the standard criteria. PLM were identified according to the WASM scoring criteria (Zucconi, et al. 2006). PLM occurring both during sleep and wakefulness were included. PLM temporally related to oxy-hemoglobin desaturation of 4% or more, indicating events of sleep apnea or hypopnea, were excluded from the analyses. Also leg movements, during which either anterior tibial EMG, oxy-hemoglobin saturation or plethysmogram signal was of insufficient quality or sleep stage could not be determined, were excluded from the study. Only leg movements not accompanied by another movement 10 seconds before or 20 seconds after the movement were included in the analysis.

Plethysmogram data and PLM event data were exported from Somnologica Studio (version 3.3.2, Medcare, Reykjavik, Iceland) into text files. Plethysmogram was analyzed with a custom script created with programming language R. Plethysmogram data was then segmented into time windows of 30 seconds (from 10 seconds before to 20 seconds after each movement). This window was chosen to avoid the inclusion of the following or previous movements in the time window. Peaks and nadirs were detected from the plethysmogram signal by calculating local minimum and maximum for each pulse wave in order to estimate PWA. Heart rate during the 30-second window was used to rule out false peaks related to movement artefacts or other disturbance in the signal. An envelope curve was created separately for the maximum and minimum values. Figure 1 shows an example of peak detection and formation of the envelope curve in the case of one single leg movement.

The 30-second time window was divided into 14 time points, located two seconds apart. Values for these time points related to individual movements were acquired from the envelope curves through interpolation. Median values of each time point were calculated for each patient. The procedure was

performed separately to the two envelope curves at the peaks and nadirs of the plethysmogram signal. The events during sleep and wakefulness were computed in separate figures.

The study was approved by the local ethical committee in Tampere, Finland. All subjects signed informed consent before participation to the study.

RESULTS

The mean age of the patients enrolled into the study was 56.6 years. Out of ten patients, seven were male. Average IRLS severity score was 22.5. Nine out of ten patients had PLM during the initial PSG. A total of 2606 leg movements were included into the final analyses, 2090 of which (80%) were during sleep. A total of 809 leg movements (24%) were detected but not analyzed due to difficult movement artefact in the plethysmogram signal or absence of the signal due to sensor failure. The total number of PLM accepted to the analysis varied from 81 to 808 per patient (Table 1).

The PWA responses to the PLM during sleep varied from patient to patient (Figure 2). A visually clear response to PLM in the plethysmogram signal was detected in four out of nine subjects. In these patients, the PWA dipped by an average of 24% (range 12-30%) from the baseline level prior to the movement during sleep and 12% (range 6-20%) during wakefulness. In cases of single movements, the dip in the PWA could be up to 50% from baseline. Four patients did not show PWA responses related to the PLM. One recording had a saturated plethysmogram signal, and therefore the presence of vasoconstriction could not be determined.

If the transient PWA responses were present during sleep, they also appeared with leg movements during nocturnal wakefulness in the same patient. On the other hand, when no responses were observed during sleep, they were absent also during wakefulness. The magnitude of the dip in the PWA, when present, was lower during wakefulness than during sleep (Figure 2).

The dip in the PWA did not herald the leg movements, but tended to start 2-10 seconds after the start of the movement. In most cases, the dip lasted for 10-20 seconds, after which the PWA returned to the baseline.

Pramipexole medication suppressed the leg movements effectively. The cortical events, such as arousals and cyclic alternating pattern, remained after the re-continuation of pramipexole. However, the transient dips in the PWA signal were suppressed (Figure 3).

DISCUSSION

The most important finding of our study is the association of transient peripheral vasoconstriction with PLM in some patients with RLS. This phenomenon has not been described before in systematic studies, and provides new evidence for the activation of the sympathetic nervous system related to PLM. The suppression of both PLM and the vasoconstriction events shows that the vascular events are related to the leg movements.

The method of PWA derived from the photoplethysmogram signal was used in our study to estimate the vasoconstriction and vasodilation in the toe. Previously, similar measurements have been performed in the finger, which is the conventional placement of the sensor (Grote, et al. 2003). In that study, the authors concluded that the PWA measurement did not reflect the forearm vascular

flow, but was a result of local peripheral blood flow regulation. The same is likely to be true in the toe. As vasoconstriction and vasodilation in the periphery are largely controlled by the sympathetic vasoconstrictor tone (Doupe, et al. 1939), the PWA responses found in our study are likely to be results of sympathetic activation related to the leg movements.

Our data indicates that the vasoconstriction response is present in some but not in all patients. Age, gender or RLS severity did not explain why the responses were present in some patients but not in others. The finding could, however, be explained by the positioning of the plethysmogram sensor. We observed that when a unilateral movement occurred in the other leg than where the sensor was attached, the response was often not present. When the movement and sensor were in the same leg, the response was more often visible. This pattern, however, was not true for all cases and therefore does not explain all the differences. Unfortunately, we did not anticipate this finding and only one oximeter with plethysmogram signal was used. Further studies with bilateral toe plethysmography should be conducted to test whether there is a difference in PWA responses between ipsilateral and contralateral legs.

In our study, pramipexole suppressed both the leg movements but left the associated cortical events unaffected. This supports the previous study reporting the same phenomenon (Manconi, et al. 2012). Our data shows, however, that the events of peripheral vasoconstriction, associated with PLM, are also suppressed by pramipexole. This suggests that these events are tightly coupled with the leg movements and not generated by the same mechanisms as the cortical arousals. Therefore, the movement-related vasoconstriction is likely to be a local event related to the muscle contraction. This is also supported by the finding that vasoconstriction is often not present in the contralateral toe during a unilateral movement. During wakefulness, muscle contraction is accompanied by vasoconstriction on the skin and vasodilation in the muscle tissue, in order to direct blood flow into

the active tissue (Johnson 1986). The vasoconstriction following leg movements serves there the purpose of redirecting blood flow to areas where perfusion might be insufficient.

Our study has several limitations. The small sample size does allow only initial descriptive analysis and studies with larger populations are needed to assess the presence and distribution of the responses in the RLS population. Another limitation is the exclusion of many movements due to movement artefacts. With an optically obtained photoplethysmogram signal, these artefacts are difficult to avoid when measurements are performed in limbs during movements. A third potential source of bias in our data is the frequent saturation of the plethysmogram signal in a part of the recordings. This may have hidden PWA responses and caused false negative findings in the recordings affected. These two issues could be addressed in future studies by measuring the plethysmogram signal with sensors based on direct measurement of the volume (Grote, et al. 2003).

As a conclusion, we described transient peripheral vasoconstriction on the skin related to the PLM in some patients with RLS. The events of vasoconstriction, unlike cortical arousals, seem to be in direct causal relationship with the leg movements, suggested by the suppression of both by pramipexole. These findings provide further evidence of the activation of sympathetic nervous system in relation to PLM, and possibly also explaining their connection with cardiovascular disease (Koo, et al. 2011).

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LEGENDS

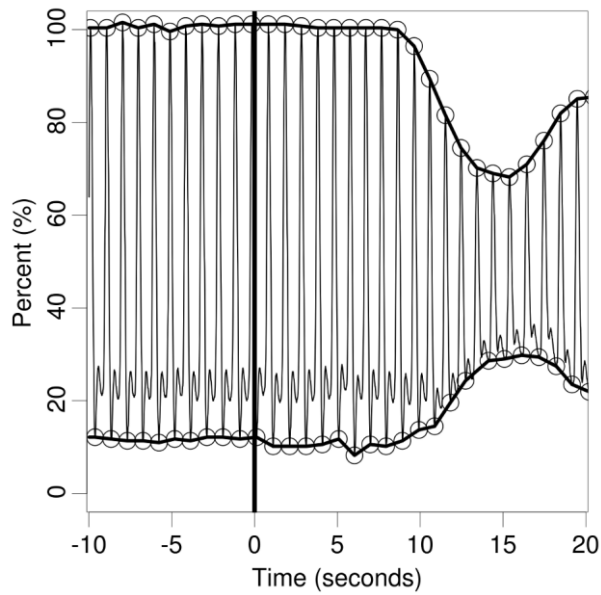


Figure 1. An example of the peak detection and the formation of the envelope curve in the analysis of the pulse-wave amplitude. The circles represent the detected peaks, whereas the two envelope curves, formed with the help of these peaks, are drawn with a black line. The y-axis is indicated as percentage of the technical maximum of the original arbitrary units of the photoplethysmograph signal. On the x-axis, the beginning of leg movement is indicated with the zero value and a vertical line. In this case, the dip starts 8 seconds after the start of the movement.

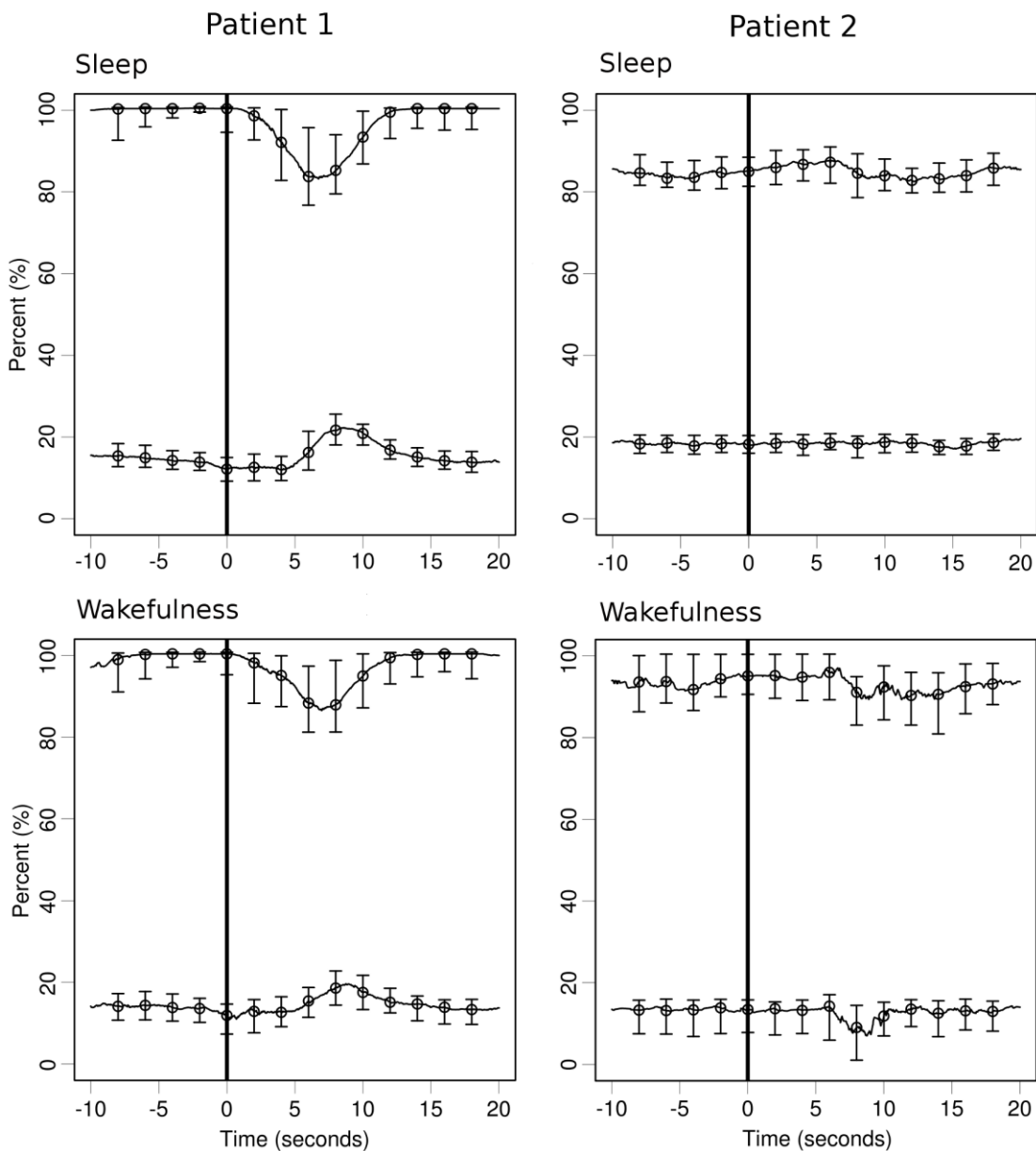


Figure 2. The median envelope curves of the plethysmogram signal are shown for two different patients during sleep and wakefulness. The first patient (left panels) showed distinct dips of the pulse-wave amplitudes after the start of the leg movements. They were present both during sleep and wakefulness. The second patient (right panels) shows less remarkable responses in both states. Values are presented as median and first and third quartile. The y-axis in these figures is indicated as percentage of the technical maximum of the photoplethysmograph signal. On the x-axis, the beginning of leg movement is indicated with the zero value and a vertical line.

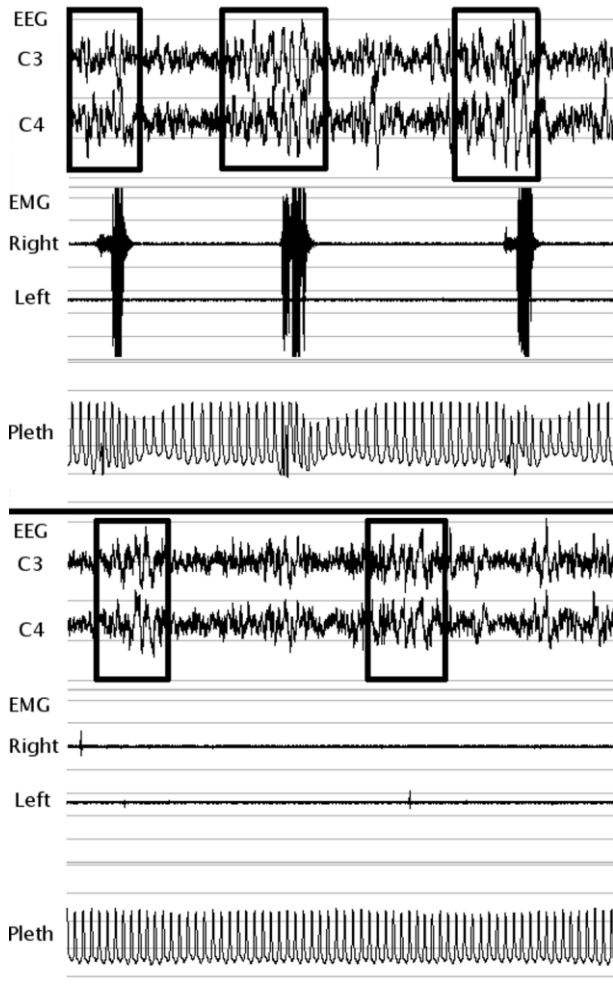


Figure 3. Periodic leg movements were accompanied by cortical arousals and dips in pulse-wave amplitude in the plethysmogram signal (left panel). After administration of pramipexole, the movements and vasoconstriction responses were suppressed but cortical arousals remained (right panel). The examples are from sleep recordings of the same patient. Displayed are two EEG channels, bilateral tibialis anterior EMG and plethysmogram (“Pleth”).

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Disconnection between Periodic Leg Movements and Cortical Arousals in Spinal Cord Injury

Aaro V. Salminen, M.Sc.^{1,2}; Mauro Manconi, M.D., Ph.D.³; Ville Rimpilä, M.Sc.^{1,2}; Teemu M. Luoto, M.D.⁴; Eerika Koskinen, M.D.⁴; Raffaele Ferri, M.D., Ph.D.⁵; Juha Öhman, M.D., Ph.D.^{1,4}; Olli Polo, M.D., Ph.D.^{1,2,6}

¹University of Tampere, School of Medicine, Tampere, Finland; ²Unesta Research Centre, Tampere, Finland; ³Sleep and Epilepsy Center, Neurocenter of the Southern Switzerland, Civic Hospital of Lugano, Lugano, Switzerland; ⁴Department of Neurosciences and Rehabilitation, Tampere University Hospital, Tampere, Finland; ⁵Sleep Research Centre, Department of Neurology IC, Oasi Research Institute (IRCCS), Troina, Italy; ⁶Department of Respiratory Medicine, Tampere University Hospital, Tampere, Finland

Objective: In this study we examine the temporal connection between periodic leg movements (PLMs) and cortical arousals, as well as the treatment effect of pramipexole, in a clinical case with spinal cord lesion.

Methods: A patient with complete cervical spinal cord injury and PLMs during sleep underwent two baseline sleep recordings, one recording with dopaminergic treatment, and one recording with adaptive servoventilation.

Results: The PLMs were temporally dissociated from cortical arousals as well as from respiratory or heart rate events. PLMs were suppressed by pramipexole and persisted after treatment of apnea.

Conclusion: The disconnection of PLMs from arousals

supports a spinal generator or peripheral trigger mechanism for PLMs. The suppression of movements by a dopamine agonist suggests that its site of action is caudal to the cervical lesion and outside of the brain. Our observation provides significant new knowledge about the pathogenesis of PLMs and warrants studies in larger populations.

Keywords: Periodic leg movements, spinal cord injury, dopamine agonist, cortical arousal, sleep apnea, case report

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There is no common agreement on the origin of periodic leg movements (PLMs) during sleep. PLMs are suppressed by dopaminergic therapy, which has led to a hypothesis of dopaminergic dysfunction in the brain¹ or in the hypothalamospinal inhibitory pathways.² PLMs are accompanied by simultaneous heart rate elevations and cortical arousals.³ However, the suppression of PLMs does not affect cortical arousals, and pharmacological reduction of cortical arousals does not affect PLMs.⁴ This may imply that PLMs and EEG arousals are simply synchronized with each other without a necessary causal relationship.

PLMs have also been described in spinal cord injury (SCI).⁵ These findings do not support the hypothesis of cerebral origin of PLMs. The movements in this subgroup of patients have not been analyzed in detail. In this study, we describe a clinical case with complete SCI in whom dopaminergic-responding PLMs are not synchronized with cortical and autonomic arousals.

REPORT OF CASE

A 35-year-old male, with a cervical SCI sustained in a car accident 17 years prior to the study, underwent 4 polysomnographic studies. The initial polysomnogram was a part of a larger project focusing on neuroimaging and respiratory functions in patients with cervical SCI. Magnetic resonance imaging confirmed the complete cervical spinal lesion

between vertebrae C3 and C5. The patient had no motor or sensory function below the C5 level. The patient was treated with tizanidine (12 mg daily) and baclofen (30 mg daily) for muscle spasticity. The patient did not suffer from RLS or daytime sleepiness.

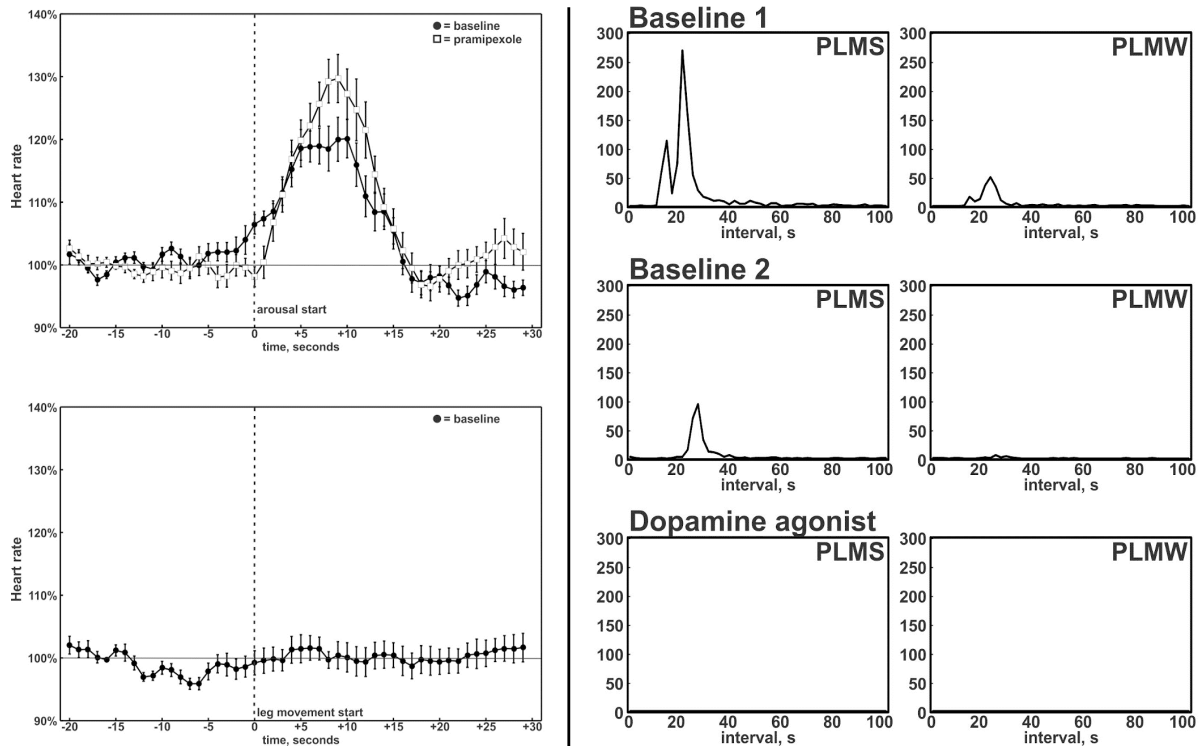
Two baseline recordings revealed excessive PLMs (PLM index 138/h and 36/h). Severe obstructive sleep apnea (apnea-hypopnea index 45.5/h and 74.5/h) was also discovered. The analysis of interval distributions of PLMs⁶ revealed a leptokurtic peak at 20-30 seconds in baseline recordings (**Figure 1**).

Cortical arousals, heart rate elevations, and respiratory events were synchronized but consistently temporally dissociated from PLMs (supplemental **Figure S1**). Increases in the heart rate were associated with the respiratory events, but were absent after PLMs (**Figure 1**). Resumption of respiration with cortical and autonomic arousals at the termination of apnea was not accompanied by PLMs.

To assess the response of the leg movements to dopamine agonists, a single test dose (0.25 mg) of pramipexole, a D3 preferential dopamine receptor agonist,⁷ was administered before a third recording. Pramipexole abolished PLMs completely. The sleep apnea was treated with adaptive servoventilation during a fourth night. The treatment resolved the sleep apnea, but PLMs persisted (PLM index 69.9/h).

The study was approved by the local ethics committee. Written informed consent was obtained from the patient.

Figure 1



Arousals resulting from events of sleep apnea were characterized by heart rate elevations (upper left panel). Heart rate elevations were not associated with PLMs (lower left panel). However, during baseline recordings, leg movements were characterized by the usual peak at 20-30 seconds in the distribution of inter-movement intervals (right panels). The peak was suppressed by pramipexole. PLMS, periodic leg movements during sleep; PLMW, periodic leg movements during wakefulness.

DISCUSSION

Our case study indicates that PLMs may occur despite a complete lesion of the motor and sensory pathways at the level of the cervical spinal cord. However, they are not accompanied by the usual synchronous cortical events or heart rate responses. Our study also demonstrates that pramipexole is able to suppress PLMs even in the absence of a brain connection. These findings support the existence of a spinal generator or a peripheral trigger of PLMs. Suppression of PLMs by pramipexole suggests that the cerebral effects of dopamine agonists are not critical for achieving the therapeutic response.

Movements in our subject fulfill the standard criteria for PLMs. Their distribution of inter-movement intervals was typical for PLMs, and they were suppressed by pramipexole. Therefore they cannot be classified as other motor disorders frequently associated with myelopathies, such as spasticity or myoclonus.

Appearance of PLMs without synchronous cortical events in a patient with an impaired upper motor neuron suggests that the origin of the movements is outside the brain. Therefore, a spinal pacemaker or a peripheral trigger of PLMs is likely. This is supported by the absence of cardiac responses to PLMs in our subject. The findings are in line with the A11 theory of the origin of PLMs.² Lack of supraspinal dopaminergic inhibition could result in activation of a pacemaker in the spinal cord to generate PLMs. Alternatively, a peripheral afferent stimulus

triggering PLMs as a spinal reflex would also be supported by our findings.

The suppression of PLMs by pramipexole suggests that also the site of action of dopamine agonists in the suppression of PLMs is located outside of the brain. Spinal cord is one candidate for the site of action, but dopamine receptors are also present outside the nervous system.

In conclusion, PLMs may be generated independently from the brain, appear in disconnection from cortical events and from autonomic activations, and can be suppressed by a dopamine agonist without connection to the brain. These findings shed new light on the pathophysiology of PLMs, suggesting a spinal generator, with or without the concurrence of peripheral triggers. SCI is an interesting model for studying the mechanism of PLMs, also allowing systematic investigations in larger populations to confirm these results.

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Address correspondence to: Aaro V. Salminen, MSc, Unesta Research Centre, Finn-Medi 6, Biokatu 14, 33520 Tampere, Finland; Tel: +358 50 345 8970; Fax: +358 3 261 7778; E-mail: aaro.salminen@unesta.fi

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SUPPLEMENTAL MATERIAL

Figure S1—Temporal disconnection between leg movements and cortical arousals is demonstrated by a sample from the first baseline sleep recording

