The global peripheral chemoreflex drive in patients with systemic sclerosis: a rebreathing and exercise study

M.K. NINABER¹, W.B.G.J. HAMERSMA¹, A.A. SCHOUFFOER²,³, E.F.A. VAN ´T WOUT¹ and J. STOLK¹

From the ¹Department of Pulmonology, ²Department of Rheumatology, Leiden University Medical Center, Leiden and ³Department of Rheumatology, HAGA Hospital The Hague, The Netherlands

Address correspondence to Maarten Ninaber, Department of Pulmonology (C3), Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands. email: m.k.ninaber@lumc.nl

Received 11 June 2014 and in revised form 27 June 2014

Summary

Background: Exercise intolerance (EI) in systemic sclerosis (SSc) is difficult to manage by the clinician. The peripheral chemoreflex drive compensates for metabolic acidosis during exercise and may be related to EI.

Aim: To assess the global peripheral chemoreflex drive (GPCD) in patients with SSc at rest and during exercise.

Methods: Consecutively tested SSc patients (n = 49) were evaluated by pulmonary function tests, carbon dioxide (CO₂) rebreathing studies and non-invasive cardiopulmonary exercise testing (CPET). Results of their CO₂ rebreathing tests were compared with those of controls (n = 32). Respiratory compensation for metabolic acidosis during CPET was defined by the occurrence of a sharp increase in minute ventilation (VdotE) and the ventilatory equivalent for CO₂ (V'E and V'CO₂) at the end of the isocapnic buffer phase. Euoxic (eVHR) and hyperoxic (hVHR) ventilatory responses to hypercapnia were measured and its difference (eVHR – hVHR) was considered to reflect the GPCD.

Results: In 45 patients with SSc, CPET results showed respiratory compensation at the occurrence of metabolic acidosis. eVHR – hVHR in patients with diffuse cutaneous SSc (dcSSc) differed significantly from that in patients with limited cutaneous SSc (lcSSc) and from that in controls (0.47 ± 0.38 (dcSSc) vs. 0.90 ± 0.77 (lcSSc) and 0.90 ± 0.49 (controls) l/min/mmHg; P = 0.04 and P = 0.03, respectively).

Conclusions: Respiratory compensation for metabolic acidosis occurred in all patients. However, the GPCD was diminished in dcSSc patients, suggesting an altered control of breathing. Its assessment may help the clinician to better understand reported EI and exertional dyspnea in dcSSc patients.

Background

Typically, progressive systemic sclerosis (SSc) may involve interstitial lung disease (ILD) and pulmonary hypertension (PH).¹ In some cases of progressive SSc, however, thoracic wall involvement may arise and manifest as an impairment in chest wall excursions caused by thickened thoracic skin, referred to as ‘sclerodermic chest wall’.² The impedance of the respiratory system is influenced by lung and chest wall compliance and respiratory flow resistance.²,³ In progressive SSc, dyspnea may arise from an increased impedance of the respiratory system caused by ILD. In SSc, ILD or limited chest wall excursions due to a thickened thoracic skin is considered to cause this increased impedance. Consequently, ventilatory impairment as a result of restriction may occur, resulting in alveolar carbon dioxide (CO₂) retention and subsequently in hypercapnic respiratory failure.²,³ It has been postulated that hypercapnic respiratory failure and exercise

© The Author 2014. Published by Oxford University Press on behalf of the Association of Physicians. All rights reserved. For Permissions, please email: journals.permissions@oup.com
intolerance (EI) may, in contrast, involve a gradual
downregulation in central and peripheral chemo-
sensitivity, resulting in slightly chronic elevated ar-
terial partial carbon dioxide pressures (PaCO₂) and
reported dyspnea during exercise.2,3 Therefore, re-
spiratory failure and EI in SSc may include not only
increased respiratory impedance (i.e. reduced
respiratory compliance and/or increased flow resist-
ance) but also a diminished peripheral chemoreflex
drive. Moreover, an absent peripheral chemoreflex
drive itself may induce an early onset of metabolic
acidosis and therefore an EI and reported exertional
dyspnea.4,5

The peripheral chemoreflex drive plays an important
role in the control of breathing.4,5 It not only
ensures oxygen homeostasis but also helps maintain
CO₂ levels at rest and during exercise.4–6 Activation
by peripheral chemoreceptors has been implicated in
ventilatory compensation for metabolic acidosis
during exercise.4–6 This ventilatory compensation is
reflected in a sharp increase in the ventilatory
equivalent for CO₂ (VE and V’CO₂) at the end of
the isocapnic buffer phase and a decrease in end-
tidal pCO₂.6

The pathophysiology of SSc is complex, involving
immune activation and widespread vascular in-
jury.7–9 Although SSc is primarily a microvascular
disorder with perivascular cellular infiltrates that
consist of macrophages, T cells and B cells, with a
predominance of CD4+ T cells, macrovascular in-
volvement has been reported as well.5,9 The carotid
bodies, the site of the peripheral chemoreflex to
oxygen, CO₂ and pH, contain a complex micro-
vascular anatomy in a macrovascular environment.
SSc-related inflammatory and fibrotic responses may
cause a diminished peripheral chemoreflex, which
may result into an increased susceptibility to EI
and consequently reported dyspnea during exer-
cise.4–6;19 We therefore hypothesized that the per-
ipheral chemoreflex drive in normocapnic SSc
patients is diminished at rest and during exercise.
We obtained CO₂ rebreathing studies in SSc patients
and healthy controls, and all SSc patients performed
a cardiopulmonary exercise test (CPET).

Patients and healthy controls

We consecutively tested 49 SSc patients referred to
an outpatient health care program. All patients were
included in the study and underwent pulmonary
function tests (PFTs), CO₂ rebreathing tests at rest
and non-invasive incremental CPET on a bicycle
according to Wasserman.6 No patients were
excluded. All tests were done in 1 day or on two
consecutive days between April 2010 and January
2012. Patients were classified as having limited cu-
taneous sclerosis (lcSSc) or diffuse cutaneous sys-
temic sclerosis (dcSSc) according to the LeRoy
criteria.10 All healthy controls performed the CO₂
rebreathing tests at rest only; PFTs were expected
to be normal.

Euoxic (eVHR) and hyperoxic (hVHR)
ventilatory response to hypercapnia at rest

CO₂ rebreathing tests were first obtained in all sub-
jects. To assess the global peripheral chemoreflex
drive (GPCD), we assessed the ventilatory response
to hypercapnia under both euoxic (eVHR) and
hVHR conditions as previously described.11
Briefly, under hyperoxia, the peripheral chemoreflex
drive is considered to be suppressed for at least
30 min.12,13 The central chemoreflex drive to hyper-
capnia can therefore best be assessed for several
minutes during hyperoxia. When the ventilatory re-
sponse to hypercapnia under euoxia is measured,
the total chemoreflex drive consists of both central
and peripheral chemoreflex drives.14–16 These
drives are then assumed to be additive.13,16 Thus,
when the inspiratory fraction of oxygen in room air
is kept constant at a level of 21%, the global con-
tribution of the peripheral chemoreceptor to total
ventilatory response to hypercapnia can be evalu-
ated by calculating eVHR minus hVHR (eVHR – hVHR).11,13–16

In SSc, increased respiratory impedance may be
present as a result of ILD, thoracic wall involvement
or increased flow resistance.2,3,17 However, because
we measured eVHR and hVHR in a single SSc pa-

tient on the same day with a limited time interval,
we did not consider the respiratory impedance to
influence the peripheral chemoreflex loop gain
eVHR – hVHR). As a result, the GPCD may be
compared between SSc patients and controls, irre-
spective of increased respiratory impedance.

Non-invasive CPET

Symptom-limited, non-invasive incremental CPET
(without arterial blood gas sampling) was next per-
formed under physician supervision in all SSc pa-

tients.18 To evaluate the presence of a peripheral

Methods

Ethics

The local Medical Ethical Committee of the
Leiden University Medical Center approved the
protocol. Written informed consent was obtained
from each participant prior to enrolment in the
study.
chemoreflex drive during exercise, we monitored minute ventilation (VdotE) and V'E and V'CO₂ continuously by breath-by-breath analysis. The anaerobic threshold was determined by use of the lowest ventilatory equivalent for oxygen (VdotE/VdotO₂) or the V-slope method whenever appropriate. Normally, when exercise continues until the occurrence of metabolic acidosis (i.e. at the end of the isocapnic buffer phase), a sharp increase in V'E and V'CO₂ provides ventilatory compensation which reflects an active peripheral chemoreflex drive (Figure 1). In all CPET results, this response was scored qualitatively as being either present or absent. Since this response is expected in all healthy controls, they did not undergo CPET.

Results

Study population characteristics

Our 49 SSc patients were extensively characterized (Table 1). LcSSc patients (n = 20) differed significantly from patients with dcSSc (n = 29) with respect to disease duration, duration of skin disease, modified Rodnan skin score, onset of Raynaud phenomenon and current and previous treatment.

Pulmonary function tests

DcSSc patients differed significantly from lcSSc patients in predicted forced vital capacity (FVC%; P = 0.034) and total lung capacity, helium dilution method (TLC-He%; P = 0.034) (Table 2). Furthermore, gas transfer (transfer factor of the lung for carbon monoxide (DLCO) single breath method) was impaired in both groups, although it was not significantly different. These parameters may indicate the presence of increased respiratory impedance and therefore ILD, PH or both.

Euoxic and hyperoxic ventilatory response to hypercapnia

In healthy controls, the mean eVHR was 3.08 ± 0.75 l/min/mmHg and differed significantly from that in dcSSc patients (1.87 ± 0.75 l/min/mmHg; P < 0.001). Similarly, the mean hVHR in controls differed significantly from that in dcSSc patients (2.46 ± 0.91 vs. 1.41 ± 0.62 l/min/mmHg; P < 0.001), but not from that in lcSSc patients (1.70 ± 0.98 l/min/mmHg; P = 0.11).

Global peripheral chemoreflex drive (eVHR – hVHR) in dcSSc patients was diminished and differed significantly from that in lcSSc patients (0.47 ± 0.38 in dcSSc vs. 0.90 ± 0.77 in lcSSc; P = 0.04) and from that in healthy controls (0.47 ± 0.38 in dcSSc vs. 0.90 ± 0.49 l/min/mmHg in healthy controls; P = 0.03). Figure 2 presents the mean (eVHR – hVHR) response in all subjects.

Furthermore, in 14 of 29 (48%) dcSSc patients, previous treatment consisted of autologous hematopoietic stem cell transplantation, which had a significant impact on the modified Rodnan skin score and on Raynaud phenomenon. In 15 dcSSc patients who were not treated with stem cells, the GPCD was significantly diminished compared with 14 stem-cell-treated dcSSc patients (0.27 ± 0.23 and 0.64 ± 0.42 l/min/mmHg, respectively, P = 0.008).

Non-invasive CPET

In total, 45 SSc patients (20 lcSSc, 25 dcSSc) reached metabolic acidosis during incremental
exercise (end of isocapnic buffer phase). Four SSc patients (all dcSSc) discontinued their exercise before reaching anaerobic threshold and reported exertional dyspnea. In all CPET results for these 45 SSc patients, ventilatory compensation to metabolic acidosis occurred by means of a sharp increase in VdotE and V'E and V'CO2 (i.e. Figure 1, data not shown).19 Within these 45 SSc patients, peak aerobic capacity (VdotO2peak), a parameter of exercise tolerance, did not differ between lcSSc and dcSSc patients (data not shown).

### Table 1 Clinical characteristics of 49 SSc patients and 32 healthy controls

<table>
<thead>
<tr>
<th></th>
<th>lcSSc n = 20</th>
<th>dcSSc n = 29</th>
<th>Controls n = 32</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, female, no. (%)</td>
<td>18 (90)</td>
<td>18 (62)</td>
<td>16 (50)</td>
<td>0.35</td>
</tr>
<tr>
<td>Age, years, mean (SD)</td>
<td>57.1 (8.8)</td>
<td>49.1 (12.2)</td>
<td>45.4 (15.8)</td>
<td>0.65</td>
</tr>
<tr>
<td>Ethnicity, no.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>19</td>
<td>22</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration, years, median (IQR)</td>
<td>10.1 (8.1)</td>
<td>4.7 (4.5)</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Skin duration, years, median (IQR)</td>
<td>11.7 (9.3)</td>
<td>6.1 (6.4)</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Onset of Raynaud phenomenon, months, median (IQR)</td>
<td>14.8 (11.2)</td>
<td>8.5 (8.9)</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>MRSS (0–51), mean (SD)</td>
<td>3.9 (4.1)</td>
<td>7.8 (6.8)</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Current treatment, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0 (0)</td>
<td>6 (19)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Prednisone</td>
<td>1 (0.04)</td>
<td>4 (13)</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Azathioprine</td>
<td>0 (0)</td>
<td>4 (13)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Previous treatment, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>0 (0)</td>
<td>14 (45)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Stem cell transplantation</td>
<td>0 (0)</td>
<td>10 (32)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>2 (8)</td>
<td>15 (48)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Prednisone</td>
<td>2 (8)</td>
<td>11 (35)</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

*aSSc, systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; dcSSc, diffuse cutaneous systemic sclerosis; SD, standard deviation; IQR, interquartile range; MRSS, modified Rodnan skin score.

bChi square, Student t-test, Mann Whitney U test or Fisher exact test where appropriate between limited and diffuse SSc.

### Table 2 Pulmonary functions tests and ventilatory responses to hypercapnia

<table>
<thead>
<tr>
<th></th>
<th>lcSSc n = 20</th>
<th>dcSSc n = 29</th>
<th>Controls n = 32</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (% pred)</td>
<td>101 (21)</td>
<td>84 (20)</td>
<td>0.034*</td>
<td></td>
</tr>
<tr>
<td>DLCOcSB (% pred)</td>
<td>63 (19)</td>
<td>57 (15)</td>
<td>0.19*</td>
<td></td>
</tr>
<tr>
<td>TLC-He (% pred)</td>
<td>91 (21)</td>
<td>80 (16)</td>
<td>0.034*</td>
<td></td>
</tr>
<tr>
<td>eVHR</td>
<td>2.59 (1.27)</td>
<td>1.87 (0.75)</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>hVHR</td>
<td>1.70 (0.98)</td>
<td>1.41 (0.62)</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>eVHR – hVHR</td>
<td>0.90 (0.77)</td>
<td>0.47 (0.38)</td>
<td>0.04***</td>
<td></td>
</tr>
</tbody>
</table>

dcSSc, diffuse cutaneous systemic sclerosis; DLCOcSB, carbon monoxide gas transfer factor corrected for hemoglobin, single breath method (mmol/min/kPa); eVHR, euoxic ventilatory response to hypercapnia (l/min/mmHg); eVHR – hVHR, global peripheral chemoreflex drive (l/min/mmHg); FVC, forced vital capacity (l); % pred, percentage predicted; hVHR, hyperoxic ventilatory response to hypercapnia (l/min/mmHg); lcSSc, limited cutaneous systemic sclerosis; TLC-He, total lung capacity, helium-dilution method (l).

*P value expressed for Student t-test between lcSSc and dcSSc.

**P value expressed for Student t-test between dcSSc and controls.

***P value expressed for Student t-test between lcSSc and controls.

Exercise (end of isocapnic buffer phase). Four SSc patients (all dcSSc) discontinued their exercise before reaching anaerobic threshold and reported exertional dyspnea. In all CPET results for these 45 SSc patients, ventilatory compensation to metabolic acidosis occurred by means of a sharp increase in VdotE and V'E and V'CO2 (i.e. Figure 1, data not shown).19 Within these 45 SSc patients, peak aerobic capacity (VdotO2peak), a parameter of exercise tolerance, did not differ between lcSSc and dcSSc patients (data not shown).

**Discussion**

In progressive SSc, the GPCD may be altered. Our results show the presence of an active peripheral...
The global peripheral chemoreflex drive in patients with systemic sclerosis

chemoreflex drive during maximal exercise in all patients with SSc, as indicated by ventilatory compensation for metabolic acidosis. However, the GPCD, as measured by the difference in eVHR and hVHR, was diminished in our dcSSc patients compared with that in healthy controls.

This is the first study on the hypercapnic ventilatory response to evaluate the peripheral chemoreflex drive in patients with SSc. In this study, global assessment of CO₂ responsiveness was used to characterize different populations. We used room air in a rebreathing bag and kept the inspired fraction of oxygen during the euoxic rebreathing test constant at a level of 21%.11 However, some considerations may apply to the methods used in this study. First, age, sex, status of menstrual phase and ethnicity all affect the ventilatory response to CO₂.20 In our study, age and sex were not significantly different between SSc patients and healthy subjects. Furthermore, the ventilatory response did not significantly differ between healthy male and female SSc patients in the eVHR and hVHR tests (data not shown). Therefore, taking these results together, we believe that these issues did not contribute significantly to differences in ventilatory responses. Second, some dcSSc patients were previously treated with high-dose cyclophosphamide and autologous hematopoietic stem cell transplantation. Such treatment was not given to patients with lcSSc and may have contributed to the variability in ventilatory responses.

We used the concept of testing the GPCD by subtracting ventilatory responses to different oxygen supplies, as previously suggested by Duffin.16 In mammals, the carotid bodies are responsible for 95% of the ventilatory response to hypoxemia21 and 30% of the response to arterial hypercapnia. In patients with resected bilateral carotid bodies, a 20–40% decrease in ventilatory response to euoxic hypercapnia is observed.19 Under euoxia, the peripheral chemoreflex drive is less active than when measured under hypoxia and considered to be 50–80% of total peripheral chemoreceptor sensitivity.14,15,19,20 In a previous study, we evaluated the peripheral chemoreflex drive in paraganglioma patients by subtracting the hVHR from the euoxic ventilatory response to CO₂.11 Furthermore, by using different levels of oxygen it was possible to determine the quantitative contribution of the chemoreceptors to ventilation at different levels of peripheral chemoreceptor stimulation.15,22 Consequently, the GPCD to CO₂ could be estimated from the difference in the euoxic and hVHR slopes.11,14–16 We therefore designated peripheral chemoreflex sensitivity under euoxia minus the hVHR ventilatory response (eVHR – hVHR) as the GPCD.

In the setting of ILD or PH, minute ventilation may be strongly influenced by increased respiratory impedance (i.e. reduced respiratory compliance and/or increased flow resistance). In our study, lcSSc and dcSSc patients differed significantly in global peripheral chemoreflex function. Moreover, since both eVHR and hVHR are measured in the same patient and within a limited time period, respiratory impedance is considered not to influence the peripheral chemoreflex loop gain (eVHR/C0hVHR). Therefore, a difference in the GPCD between lcSSc and dcSSc patients may be derived from our rebreathing studies.

In addition to its measurement during rebreathing studies, the peripheral chemoreflex function can be assessed during exercise.6,19 When the exercise work rate is high enough to produce metabolic acidosis, the increase in ventilatory response is only—and strongly—mediated by the peripheral chemoreceptors.6 Thus, in the setting of an absent carotid body function, respiratory compensation for metabolic acidosis does not occur.6,19 In all of our SSc patients, including dcSSc patients, a ventilatory compensatory response at the end of the isocapnic buffer phase occurred as a result of the presence of a peripheral chemoreflex drive. However, quantitatively, as our results from the rebreathing studies indicate, a diminished ventilatory response is present in dcSSc patients compared with that in healthy controls, suggesting an altered GPCD in the absence of metabolic acidosis.

Our results may be explained by two mechanisms. First, SSc is considered to be an inflammatory disease.8,9 Anti-inflammatory treatment by autologous stem cell transplantation may have reduced the level of inflammation in carotid bodies, as indicated by our results in dcSSc patients. In dcSSc...
patients treated with autologous hematopoietic stem cell transplantation, a significant difference was present in the GPCD compared with that in dcSSc patients without such treatment. Therefore, this drive may have been altered positively by stem cell transplantation, suggesting an impact on carotid body vascularization and consequently its function. Second, widespread atherosclerosis is present not only in lcSSc patients, but also in dcSSc patients, and may involve the carotid arteries and bodies.\(^7\)\(^8\) We did not, however, measure carotid artery intima thickness. Furthermore, serum lipid spectrum was not significantly different between lcSSc and dcSSc patients.

**Conclusions**

In summary, our results show that in all SSc patients, ventilatory compensation for metabolic acidosis occurs as a result of an active peripheral chemoreflex drive. The GPCD was, however, diminished in dcSSc patients compared with that in healthy controls, suggesting an altered chemoreflex control of breathing in these patients. This may help the clinician to better understand reported EI and exertional dyspnea in dcSSc patients.

**Conflict of interest:** None declared.

**References**