Analysis of autonomic modulation after an acute session of resistance exercise at different intensities in chronic obstructive pulmonary disease patients

Purpose: Physical exercises are employed as part of the treatment of patients with chronic obstructive pulmonary disease (COPD); however, information regarding cardiac autonomic modulation after an acute session of resistance exercise (RE) is unknown. The aim of this study was to evaluate the cardiac autonomic modulation, via heart rate variability after an acute session of RE applied at different intensities in COPD patients.

Patients and methods: Twelve COPD patients underwent an acute session of RE with an intensity of 60% and another of 90% of the one repetition maximum test. For analysis of autonomic modulation, heart rate was recorded beat-by-beat for 20 minutes at rest and after the training session. Heart rate variability indexes were obtained in the time and frequency domains for the assessment of autonomic modulation.

Results: Regardless of exercise intensity, RE acute sessions influenced the autonomic modulation when the recovery period was compared with the baseline. An increase in standard deviation of normal to normal RR intervals was observed throughout recovery time after the RE, as compared to baseline in both protocols: 60% and 90% of the one repetition maximum test. The spectral component of low frequency index (ms) was higher throughout recovery when compared to baseline in both protocols. The same was also observed in the spectral component of high frequency index (ms) for the protocols of 60% and 90%.

Conclusion: RE sessions impact on the autonomic modulation of COPD patients by promoting differences in the recovery period compared to baseline, regardless of the intensity of the exercise performed.

Keywords: heart rate variability, autonomic nervous system, sympathetic nervous system, parasympathetic nervous system, physical exercise

Introduction

Chronic obstructive pulmonary disease (COPD), a treatable and preventable disease, is characterized by airflow limitation that is usually progressive and associated with chronic airway inflammatory response.1 This disease has some extra-pulmonary effects that may contribute to its severity.2

One of the systemic manifestations that affect these patients is related to cardiac autonomic modulation. Patients with COPD have changes in this modulation that play an important role in the morbidity and mortality of these patients.3 These changes result in reduced heart rate variability (HRV), which provides a noninvasive measure to evaluate modulation of the autonomic nervous system,4 describing the

Juliana Nicolino1
Dionei Ramos1
Marceli Rocha Leite1
Fernanda Maria Machado Rodrigues1
Bruna Spolador de Alencar Silva1
Guilherme Yassuyuki Tacao1
Alessandra Choqueta de Toledo2
Luiz Carlos Marques Vanderlei1
Ercy Mara Cipulo Ramos1
1Department of Physiotherapy,
Paulista State University (UNESP),
Presidente Prudente, São Paulo, Brazil;
2Department of Pathology, School of Medicine of the University of São Paulo, São Paulo, Brazil

Correspondence: Ercy Mara Cipulo Ramos
Department of Physiotherapy,
Paulista State University, Rua Roberto Simonsen 305, Presidente Prudente,
São Paulo, CEP 19060-900, Brazil
Tel +55 18 3221 4818
Fax +55 18 3221 8212
Email ercy@fct.unesp.br
oscillations between consecutive heart beats (RR intervals), and its application in clinical practice has increased considerably. Muscle sympathetic nerve activity is higher in COPD patients compared with healthy individuals. There is evidence of enhanced sympathetic nerve traffic, elevated catecholamines, and an activated renin-angiotensin system in these individuals.

HRV has been investigated in several pathological conditions such as hypertension, myocardial infarction, coronary artery disease, diabetes, stroke, and obstructive sleep apnea. It is known that decreased HRV is considered an independent risk factor for mortality in healthy people, especially after acute myocardial infarction and is related to adrenergic hyperactivity and decreased parasympathetic activity. Furthermore, HRV has also been used to investigate physiological changes such as those associated with the execution of exercise.

Exercise promotes important changes in the functioning of the cardiovascular system and its autonomic adjustment mechanisms. In healthy subjects, performing exercise has been associated with decreased parasympathetic and increased sympathetic modulation and, after its performance, the cardiac sympathetic modulation remains increased and the parasympathetic reduced. In COPD patients undergoing exercise, cardiac autonomic modulation has been scarcely studied, especially in the post-exercise period. Understanding these responses may have significant clinical importance in these patients.

Regarding physical exercises in COPD patients, resistance exercise (RE) has been considered an important component in pulmonary rehabilitation since these patients have skeletal muscle dysfunction that is associated with limited exercise tolerance, fatigue, and dyspnea which are considered negative prognostic factors in COPD.

Therefore, considering that HRV has proven to be a clinically valuable tool for evaluation of neurological control of the heart, the scarcity of information regarding cardiac autonomic modulation in the post-exercise period, and considering that physiotherapy utilizes exercise programs as part of COPD patient treatment, the aim of this study was to evaluate the cardiac autonomic modulation through HRV after an acute session of RE at different intensities in COPD patients.

We hypothesize that the execution of an acute session of RE in COPD patients modifies autonomic modulation after its completion, and that these responses are dependent on the intensity of the exercise performed. It is hoped that this study will contribute relevant information about the impact of RE on the autonomic modulation of COPD patients.

Methods
Subjects
For the realization of this study, data from 12 COPD patients were analyzed. To participate in the study the patients had to have undergone spirometry and received a medical diagnosis of the disease, as recommended by the Global Initiative for Obstructive Lung Disease, and not have the following characteristics: smoking, home oxygen therapy use, participation in another physical training program prior to this study, reported presence of heart co-morbidities that prevented the execution of the experimental protocol, musculoskeletal disorders and alteration or addition of medication that affects the autonomic nervous system.

All procedures of this study were approved by the Ethics Committee of the Institution (CAAE: 00849812.0.0000.5402) and all patients signed an informed consent form.

Study design
The experimental protocol comprised an initial assessment and the assessment of autonomic function against RE of 60% and 90% of the one repetition maximum test (1RM), which was performed on subsequent days. The initial assessment consisted of volunteer identification, anthropometric measurements, lung function evaluation by spirometry test, and 1RM.

For all visits, patients were instructed to: avoid consuming caffeine 24 hours before the procedures; eat a light meal 2 hours before testing, abstain from alcoholic beverages for at least 4 hours, have a good night’s sleep (7–8 hours), avoid strenuous physical exercise the day before the evaluations, and be dressed in comfortable and appropriate clothing for exercise performance.

Spirometry
Lung function test was performed using a spirometer (Spirobank-MIR3.6; MIR, Rome, Italy), according to the guidelines of the American Thoracic Society and European Respiratory Society. Normality values were related to the Brazilian population.

1RM
The 1RM test was performed before RE sessions, to determine the workload to be executed. Prior to this test the correct execution of the exercise was demonstrated to the patients in order to avoid errors in its performance. Patients
underwent specific warming-up before the test and for its realization an initial workload of 20% of body weight for lower, and 5% of body weight for upper limbs was set. For the determination of 1RM, subjects had three to five attempts at intervals ranging between 3 to 5 minutes (min). The test was completed when the patient reached the load that caused mechanical failure of execution, and the final load at which they managed to perform the exercise without mechanical failure was established as a maximum load. All tests were supervised by the same evaluator.30

The movements tested to determine the 1RM and, thereafter, were: knee flexion, knee extension, shoulder flexion, shoulder abduction, and elbow flexion.

REs

The RE series was performed for 50 min on weight training equipment (Ipiranga pulley system; Hard Academy, São Paulo, Brazil) regulated in accordance with the proper positioning of the patient for the correct execution of the exercises. The movements during the exercises were: knee flexion, knee extension, shoulder flexion, shoulder abduction, and elbow flexion.

Three series of ten repetitions of each exercise, with intervals of 1 min of rest between each series, at both intensities of 60% and 90% of 1RM were performed. The implementation of protocols of 60% and 90% was performed randomly and with an interval of 48 hours. To control, blood pressure, heart rate (HR), respiratory rate, oxygen saturation, and degree of dyspnea were noted before and after exercise.

HRV

For the HRV analysis, patients’ HR was recorded beat-by-beat using a Polar S810i HR monitor (Polar Electro, Kempele, Finland), a previously validated device for capturing the beat-to-beat HR and its utilization for HRV analysis.31,32

After the explanation of the data collection procedures, an elastic catchment strap was placed on the chest of the volunteer, at the height of the xiphoid process, and the HR receiver was attached to their wrist. Initially, an HR reading for each patient was taken in the sitting position for 20 min and another after realization of the RE. The HRV assessment was performed on 2 separate days, one day at 60% and another at 90% of 1RM intensity for each exercise.

For HRV indexes analysis, 256 consecutive RR intervals were selected for each time the HRV assessed, 5 minutes more stable from tracings, which were subjected to digital filtering by Polar Precision Performance SW software (version 4.01.029) supplemented by manual filtering to eliminate ectopic premature beats and artifacts, and only series with more than 95% sinus beats were included in the study.21,22,33

Kubios software version 2.0 (MathWorks, Natick, MA, USA)34 was used to calculate HRV indexes in the time and frequency domains. The calculated indexes in the time domain were the following: standard deviation of normal to normal RR intervals (SDNN [ms]) and root mean square of differences between adjacent normal RR intervals in a time interval (RMSSD [ms]).22,35 For HRV analysis in the frequency domain, we used the spectral components of low frequency (LF: 0.04 to 0.15 Hz) and high frequency (HF: 0.15 to 0.4 Hz) frequency, in normalized units and milliseconds, in addition to the LF/HF ratio.4,22,35

The HRV indexes were measured at the following times: baseline/rest, immediately after sessions, and at 5, 10, and 15 min after.

Statistical analysis

For the population profile data analysis, a descriptive statistical method was used and the results were presented with mean, standard deviations, and minimum and maximum values. Data normality was assessed by the Shapiro–Wilk test.

Comparisons of HRV indices among protocols (60% and 90% of 1RM) and moments (baseline, immediately after, 5, 10, and 15 min after) were made by the technique of two-way analysis of variance for repeated measures model. The repeated measurement data were checked for violations of sphericity using Mauchly’s test and the Greenhouse-Geisser correction was used when sphericity was violated.

For moments analysis (basal versus immediately after, 5, 10, and 15 min after) Bonferroni post-test for parametric distribution or Dunnet’s post-test for nonparametric distribution was used. Statistical significance was set at 5% for all analyses and calculations were performed using the software SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

The patients’ anthropometric characteristics, their spirometric values, and their basal blood pressure values are described in Table 1.

All patients who participated in the study made use of a bronchodilator. In addition, 64% used angiotensin receptor antagonists, 9% diuretics, 27% hypolipidemics, 9% anti-inflammatories, and 9% anti-allergic agents.

Table 2 presents the HR and HRV indexes in the time domain at all evaluated moments. No significant
differences in HR and RMSSD and SDNN between the protocols were observed at all analyzed time points ($P>0.05$).

No differences were found in HR and RMSSD index when comparing all times after the exercise session compared to baseline values both at 60% and 90% of 1RM. It was noted that SDNN (ms) was significantly higher in every moment after RE compared to baseline for both 60% and 90% of 1RM.

The HRV indexes in the frequency domain can be seen in Table 3. No significant differences in rates between the protocols were observed at all analyzed time points ($P>0.05$).

Regarding the comparison between moments, it was observed that the LF (ms) index was higher at all times of recovery when compared to rest in both 60% and 90% of 1RM protocols. The same was observed for the HF index (ms) also in both protocols.

### Discussion

The results obtained in this study demonstrated that acute sessions of RE influenced HRV indexes regarding the analyzed moment, since the differences were observed in the recovery period compared to baseline. Furthermore, there was no difference between protocols showing that, regardless of the intensity of the performed exercise, the recovery of autonomic modulation in these patients is similar.

Several studies have described the behavior of HRV after aerobic exercise, but few show the response of autonomic function after RE. In those that evaluated HRV after RE, the authors found an increased sympathetic activity and decreased parasympathetic activity during the post-exercise recovery period. In addition, there are no studies that evaluate autonomic modulation during recovery in patients with COPD undergoing REs and the influence of exercise intensity in this response.

The SDNN index, which reflects the overall variability, displayed a significant increase in moments of recovery compared to baseline. During the recovery from exercise period, the initial return of HR to baseline occurs primarily due to parasympathetic reactivation. With the cessation of exercise, there is a loss of central command, and the baroreflex activation and other mechanisms contribute to the increase in parasympathetic activity. This parasympathetic reactivation promotes an increase in RR intervals' variation, which may be associated with SDNN index increase.

Increased sympathetic activity after RE has been described in the literature. A study by Lima et al, that evaluated the acute effects of strength exercise on HRV in young people, showed that when they performed the session with 70% of 1RM there was an increase in the LF index in the recovery period compared to pre-intervention. These findings corroborate the

### Table 1: Characterization of COPD patients

<table>
<thead>
<tr>
<th>Anthropometric data</th>
<th>Mean ± SD</th>
<th>Minimum/maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>66±10</td>
<td>[45–75]</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>7/5</td>
<td></td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.61±1.0</td>
<td>[1.4–1.7]</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>23±3</td>
<td>[19.7–31.2]</td>
</tr>
<tr>
<td><strong>Spirometric values</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁ (%)</td>
<td>42±18</td>
<td>[29–74]</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>42±13</td>
<td>[23–66]</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122±10</td>
<td>[110–140]</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79±5</td>
<td>[70–90]</td>
</tr>
</tbody>
</table>

**Abbreviations:** COPD, chronic obstructive pulmonary disease; SD, standard deviation; M, male; F, female; BMI, body mass index; FEV₁, forced expiratory volume in first second; FVC, forced vital capacity; SBP, systolic blood pressure; DBP, diastolic blood pressure.

### Table 2: Values of HR and of HRV indexes in the time domain, in evaluated moments, expressed as mean and standard deviation

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR 60%</td>
<td>77.0±4.7 (76.0)</td>
<td>86.2±9.0 (88.0)</td>
<td>76.3±7.9 (76.0)</td>
<td>73.3±6.1 (75.0)</td>
<td>72.3±6.8 (74.0)</td>
</tr>
<tr>
<td>90%</td>
<td>77.0±10.6 (77.0)</td>
<td>88.8±12.4 (89.0)</td>
<td>77.8±10.8 (80.0)</td>
<td>75.0±5.8 (76.0)</td>
<td>74.8±9.6 (72.0)</td>
</tr>
<tr>
<td>SDNN 60%</td>
<td>19.7±7.7 (17.8)</td>
<td>49.7±24.3 (45.1)*</td>
<td>27.4±9.1 (25.8)*</td>
<td>25.4±8.4 (22.8)*</td>
<td>27.6±11.9 (23.9)*</td>
</tr>
<tr>
<td>90%</td>
<td>20.0±8.9 (17.0)</td>
<td>47.9±24.7 (52.0)*</td>
<td>25.5±9.5 (27.1)*</td>
<td>26.3±8.4 (26.1)*</td>
<td>25.5±9.4 (23.1)*</td>
</tr>
<tr>
<td>RMSSD 60%</td>
<td>13.3±7.1 (12.0)</td>
<td>17.5±8.7 (15.8)</td>
<td>15.7±9.2 (13.8)</td>
<td>15.3±7.4 (13.9)</td>
<td>15.2±9.3 (12.9)</td>
</tr>
<tr>
<td>90%</td>
<td>15.3±8.0 (14.3)</td>
<td>17.5±9.6 (16.8)</td>
<td>17.0±6.1 (18.6)</td>
<td>15.8±6.0 (15.3)</td>
<td>15.8±8.0 (14.7)</td>
</tr>
</tbody>
</table>

**Notes:** Mean ± SD (Median); *statistical difference compared to baseline ($P<0.05$); I= immediately after training, 5=5 min after session, 10=10 min after session, 15=15 min after session.

**Abbreviations:** HR, heart rate; HRV, heart rate variability; SDNN, standard deviation of normal to normal RR intervals; RMSSD, root mean square of differences between adjacent normal RR intervals in a time interval; SD, standard deviation; min, minutes.
present study, since the LF (ms) index remained raised after RE at both 60% and 90% of 1RM intensities.

Regarding the parasympathetic component in the recovery period of RE, Heffner et al showed that 25 min after the cessation of exercise, the HR index remained reduced in healthy subjects.\textsuperscript{7,50,51} The same happened in the Oliveira et al study, in which HRV was assessed immediately after the cessation of exercise in healthy men who underwent 12 months of RE.\textsuperscript{44}

The data from this study do not confirm these findings since the HF (ms) index remained raised throughout the period of recovery from exercise at both 60% and 90% of 1RM in COPD patients. A study by Javorka et al,\textsuperscript{42} which evaluated HRV in healthy men after the step test, which assesses functional capacity, showed a marked reduction in the HF index during exercise and an increase during recovery, indicating parasympathetic reactivation after exercise. The authors also found a gradual increase in the LF index during recovery and suggest that the LF index is directly influenced by changes in parasympathetic activity, through changes in vagal activity that cause fluctuations in the LF band, or indirectly, by changes in baroreflex sensitivity.

Using HRV, Gonzalez-Camarena et al\textsuperscript{46} demonstrated that unlike the parasympathetic withdrawal seen during dynamic exercise, there may be an increase in vagal modulation during static exercise (resistance) without any change in LF/HF. Thus it is possible that modulation in the neuronal sinoatrial node after RE may also be different.\textsuperscript{23}

The LF/HF ratio has been shown to increase from 20 to 30 min after exercise and may remain elevated for up to 60 min post-exercise,\textsuperscript{23,45,46} suggesting a state of sympathetic predominance.\textsuperscript{7,50} In the present study, although there was no statistical difference in LF/HF ratio between the periods evaluated, we observed an increase in this index both at 60% and 90% of 1RM after RE compared to baseline.

The results also showed that regardless of the intensity of the exercises performed, autonomic modulation recovery is similar, indicating that the autonomic response after RE at 60% of 1RM is the same as at 90% of 1RM in these patients. Using diastolic blood pressure, Gurjão et al\textsuperscript{49} showed that in normotensive women different intensities of RE did not influence this parameter. COPD patients have autonomic dysfunction that promotes reduced HRV compared with healthy subjects.\textsuperscript{7,50,51} This autonomic dysfunction may, at least in part, explain the absence of differences in the autonomic modulation recovery after RE performed with intensity equivalent to 60% and 90% of 1RM.

This study has some limitations that should be taken into consideration. The absence of a healthy control group should be mentioned, which could have helped determine whether the responses obtained from COPD patients differ from normal results. Another limitation is related to the impossibility of HRV analysis during the performance of the RE series, which could provide important information for understanding the changes in autonomic modulation during the recovery period. During the performance of the RE series, rest intervals of 

---

**Table 3 Values of HRV indexes in the frequency domain, in evaluated moments, expressed as mean and standard deviation**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>65.0±79.2</td>
<td>198.0±200.7 (124.5)*</td>
<td>266.3±228.2 (198.0)*</td>
<td>205.8±172.1 (141.0)*</td>
<td>236.8±235 (130.0)*</td>
</tr>
<tr>
<td>90%</td>
<td>44.9±71.5</td>
<td>293.1±383.6 (170.0)*</td>
<td>303.7±346.4 (189.0)*</td>
<td>183.4±123.9 (146.0)*</td>
<td>272.3±247.9 (287.0)*</td>
</tr>
<tr>
<td>LF nu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>69.4±15.7</td>
<td>67.4±18.0 (66.2)</td>
<td>78.6±12.7 (71.1)</td>
<td>70.5±15.5 (70.0)</td>
<td>73.8±12.6 (74.5)</td>
</tr>
<tr>
<td>90%</td>
<td>64.2±24.3</td>
<td>66.8±22.5 (68.7)</td>
<td>69.9±22.9 (74.8)</td>
<td>68.3±18.9 (71.0)</td>
<td>71.5±22.0 (78.0)</td>
</tr>
<tr>
<td>HF ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>19.1±18.1</td>
<td>82.6±48.2 (64.5)*</td>
<td>86.6±16.1 (49.5)*</td>
<td>75.3±61.5 (57.0)*</td>
<td>106.8±138.1 (54.5)*</td>
</tr>
<tr>
<td>90%</td>
<td>21.5±29.1</td>
<td>113.3±128.9 (62.0)*</td>
<td>99.9±68.1 (102.5)*</td>
<td>84.6±51.6 (77.5)*</td>
<td>90.0±103.7 (55.5)*</td>
</tr>
<tr>
<td>HF nu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>30.1±15.7</td>
<td>32.5±18.0 (33.7)</td>
<td>21.3±12.7 (22.9)</td>
<td>29.4±15.5 (29.9)</td>
<td>26.1±12.6 (25.4)</td>
</tr>
<tr>
<td>90%</td>
<td>35.7±24.3</td>
<td>33.1±22.5 (31.3)</td>
<td>30.0±22.9 (25.2)</td>
<td>31.5±18.9 (28.9)</td>
<td>28.4±22.0 (21.9)</td>
</tr>
<tr>
<td>LF/HF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>3.2±2.3</td>
<td>3.1±2.4 (1.9)</td>
<td>7.1±6.8 (4.0)</td>
<td>4.9±7.4 (2.3)</td>
<td>4.0±4.1 (2.4)</td>
</tr>
<tr>
<td>90%</td>
<td>3.3±2.9</td>
<td>5.1±6.5 (2.2)</td>
<td>53.7±168.6 (3.5)</td>
<td>3.2±2.8 (2.4)</td>
<td>4.5±2.9 (3.7)</td>
</tr>
</tbody>
</table>

**Notes:** Mean ± SD (Median); *statistical difference compared to baseline (P<0.05); I= immediately after session, 5=5 min after session, 10=10 min after session; 15=15 min after session.

**Abbreviations:** HRV, heart rate variability; nu, normalized units; LF, low frequency spectral component; HF, high frequency spectral component; min, minutes; SD, standard deviation.
1 min between each repetition were given, which generated oscillations in HR preventing proper analysis of HRV. Additionally, while capturing HR, the patients’ respiratory rate was not controlled, which may have influenced the HF index.

Considering the importance of the theme presented, other studies are being conducted to evaluate the acute responses to resistance and aerobic exercise after physical training programs. Furthermore, research into the impact of different physical training protocols on autonomic modulation and clinical, biochemical, and functional variables in these individuals is being conducted. Studies of this nature can enrich knowledge in the area of exercise physiology in patients with COPD.

Given the importance of understanding the autonomic behavior after RE in COPD patients, who suffer from autonomic dysfunction, which is considered a risk factor associated with mortality, this study represents a useful clinical tool for professionals in the area since the results indicate that HRV indexes, a noninvasive and inexpensive measurement, can aid both in evaluating the clinical manifestations of the disease and monitoring therapeutic procedures performed with these patients. Furthermore, there is no evidence of the impact of post-exercise autonomic modulation on mortality in COPD and others diseases.

**Conclusion**

From the results obtained from this study, it is concluded that acute sessions of RE impact on autonomic modulation in COPD patients by promoting differences in the recovery period compared to baseline. However these differences are irrespective of the intensity of exercise performed.

**Acknowledgment**

This work was supported by the following Brazilian scientific agency: The São Paulo Research Foundation (FAPESP).

**Disclosure**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**References**


