Trunk stabilization estimated using pseudorandom force perturbations, a reliability study

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ABSTRACT

Measurement of the quality of trunk stabilization is of great interest to identify its role in first occurrence, recurrence or persistence of low-back pain (LBP). Our research group has developed and validated a method to quantify intrinsic and reflex contributions to trunk stabilization from the frequency response function (FRF) of thorax movement and trunk extensor EMG to perturbations applied by a linear actuator. However, the reliability of this method is still unknown. Therefore, the purpose of this study was to investigate the between-day reliability of trunk FRFs in healthy subjects and LBP patients. The test–retest ICC's in patients were substantial for both admittance and reflex gains (ICC3,1 > 0.73 and 0.67). In healthy subjects, the reliability of admittance gain was also substantial (ICC3,1 0.66), but the reliability of the reflexive gain was only moderate (ICC3,1 0.44). Although sample sizes were limited (13 healthy subjects and 18 LBP patients), these results show that trunk stabilization can be measured reliably, and represent a promising step towards using this method in further research in LBP patients.

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1. Introduction

Trunk stabilization is needed to maintain control over trunk posture and movements during daily life activities (MacKinnon and Winter, 1993; van der Burg et al., 2005). Trunk stabilization is dependent on both active (muscular) and passive (osteoligamentous) structures and it has been suggested that low-back pain (LBP) might cause impaired trunk stabilization (van Dieen et al., 2003; Panjabi, 1992), which in turn might contribute to persistence or recurrence of LBP (Hodges and Moseley, 2003; MacDonald et al., 2009). It has also been suggested that poor trunk stabilization could be a predictive factor or even primary cause of LBP (Cholewiczki et al., 2005). Therefore, measurement of the quality of trunk stabilization is of great interest to identify its role in the first occurrence, recurrence or persistence of LBP.

Specifically, several studies have indicated longer reflex delays after an external mechanical perturbation of trunk posture in LBP patients than in controls (Magnusson et al., 1996; Radebold et al., 2000). In apparent contrast, higher trunk stiffness, i.e. a higher mechanical resistance to such perturbations, has also been reported (Hodges et al., 2009). In fact, increased trunk intrinsic stiffness could explain the longer delays found, as with increased intrinsic stiffness the same mechanical disturbance will cause a smaller and slower deviation of trunk posture. This could result in a longer apparent reflex delay, caused by the smaller deviations in combination with thresholds of sensors signaling these deviations or even in the method detecting the responses, while the true neural delay could be unaffected.

Protocols to properly identify trunk stabilization (in terms of intrinsic stiffness and reflexive responses) must be well standardized and reliable to determine their clinical relevance and to support their use as clinical outcome measures. Many methods to measure trunk stabilization have been described in the literature, but documentation on the reliability of these methods is sparse (Maaswinkel et al., submitted for publication). Hendershot et al. (2012) performed a reliability study on a method that used pseudorandom position-controlled perturbations and found high within-day and moderate to fair between-day intraclass correlation coefficients (ICC's) for trunk stiffness (0.90 and 0.67 respectively) and reflex gain (0.85 and 0.37 respectively). A potential problem with the position controlled perturbations, is that the subject is unable to exert any influence over the resulting displacement and will therefore not be motivated to resist. It has been observed in former studies on upper extremity control that subjects reduce their efforts to resist position controlled perturbations after several seconds (de Vlught et al., 2003a, 2003b).
In contrast, pseudorandom force perturbations do not have this drawback and require the subject to actively resist the perturbation during the entire trial. Our research group has developed and validated a method to assess both the intrinsic and reflexive component of trunk stabilization by applying thorax perturbations with a linear actuator while subjects are restrained at the pelvis in a kneeling-seated position (van Drunen et al., 2013). However, the reliability of this method is still unknown. Reliability might be influenced by LBP because of possible variability over time in motor control impairments in LBP (Granata et al., 2004). Therefore, the purpose of this study was to investigate the between-day reliability of a pseudorandom force perturbation method to measure trunk stability in both healthy subjects and in LBP patients.

2. Methods

2.1. Subjects

Thirteen healthy subjects (5 males, age range 22–28 years, mean mass: 74 kg (± 13 kg)) and 18 patients with LBP (10 males, age range 29–69 years, mean mass: 89 kg (± 23 kg)) participated in this reliability study. All participants met the following inclusion and exclusion criteria; the healthy subjects did not have LBP in the year prior to the experiments. The group of patients suffered from non-specific LBP, or LBP following back surgery, for at least six weeks. Fusions, Prostheses or other operations that cause substantial anatomical changes were excluded. Subjects had no radicular pain caused by lumbar nerve root compression or a hernia nucleus pulposi, nor did they have any neurological disorders that might interfere with trunk control (e.g. Cerebro Vascular Accident, Multiple Sclerosis or Parkinson’s disease). All participants read and signed an informed consent form prior to the experiment according to the guidelines of the medical ethical committee of VU Medical Center Amsterdam.

2.2. Protocol

To assess the test-retest reliability, two separate measurements were performed following the procedure described below. The time between repeated measurements for healthy subjects was 1–3 days. Patients repeated the protocol after 1–2 weeks. This was done in order to decrease burden and to prevent influence of possible muscle-soreness after the first measurement.

The experimental setup was similar to previous studies (van Drunen et al., 2013; Maaswinkel et al., 2015). The participants were positioned in a semi-kneeling-seated posture with their pelvis restrained (Fig. 1). The subjects were blindfolded to prevent visual feedback and crossed their arms during the trials. During trials, a ventral force perturbation was applied at the T10 level of the spinous process by a magnetically driven linear actuator (Servotube STR2510S Forcer and Thrustrod TRB25-1380, Copley Controls, USA). A thermoplastic patch (4 x 4 cm²) was placed between the device and the subject for comfort and better force transfer. Each subject was instructed to ‘sit as still as possible’ during the perturbations (resist-task). The patients performed additional trials in which they were asked to ‘Relax, but sit upright’ (relax-task). Each condition was repeated three times.

The same pseudorandom force perturbation signal was used for each participant and for both consecutive assessments and consisted of a pseudorandom dynamic force disturbance of ≤ 35 N combined with a 60 N baseline preload. The dynamic disturbance was a crested multi-sine (sum of sine waves) (Pintelon and Schoukens, 2001) that contained 18 logarithmically distributed paired frequencies within a bandwidth of 0.2–15 Hz (Fig. 1). Power above 4 Hz was restricted to 40% to reduce adaptive behavior to high frequency content (Mugge et al., 2007). Each run lasted 50 s, consisting of a 3 s ramp force increase to the 60 N load level, a two second stationary load, a start-up period to reduce transient behavior (the last 5 s of the dynamic disturbance), and twice a 20 s dynamic disturbance.

After each trial, patients were asked for their momentary pain using a 10-point numeric pain rating scale (NPRS) (Freyd, 1923). Additionally, patients filled in a 7-days pain diary, using a 10-point NPRS, prior to both measurements. Both momentary pain and pain-diary scores were used for LBP-identification (NPRS-score > 0) and to assess the stability of pain between the two measurements. To correct for possible changes in severity between consecutive assessments of known prognostic factors in patients with low back pain (i.e., illness beliefs, fear of movement, catastrophizing, depression and anxiety), the Oswestry Low Back Pain Questionnaire (Fairbank et al., 1980), Back beliefs questionnaire (Symonds et al., 2007)
were log-transformed. Sphericity was checked with Mauchly’s test and if the

time between the two measurements in patients, a paired samples

test was used. The cross-spectra were averaged across subjects. The cross-spectral densities were only evaluated at the frequencies 2.5. Statistics

time between the Fourier transformed force-perturbation (\(F_F(f)\)) and the contact force (\(F_C(f)\)) (interaction with the subject) and EMG (\(e(f)\)) respectively. The cross-spectral densities were only evaluated at the frequencies containing power in the force perturbation. The cross-spectra were averaged across the 6 time segments per task (three trials each containing two segments of 20 s) and over 2 adjacent frequency points to improve estimates and reduce noise (Jenkins and Watts, 1969). Finally, the cross-spectra between EMG and force perturbations were averaged over the left and right muscles. The coherence of the admittance and reflexes associated with \(r_{\text{adm}}^2\) and \(r_{\text{exes}}^2\) was derived as:

\[
\frac{\left| S_{Y_F}(f) \right|^2}{S_{Y_C}(f) S_{X_C}(f)}
\]

\[
\frac{\left| S_{Y_C}(f) \right|^2}{S_{X_C}(f) S_{X_C}(f)}
\]

Coherence ranges from zero to one, where a coherence of one reflects a perfect, noise-free relation between input and output. Since spectral densities were averaged over 12 points, a coherence greater than 0.24 is significant with \(P < 0.05\) (Halliday et al., 1995).

2.5. Statistics

To test for any significant differences in the questionnaires and pain scores between the two measurements in patients, a paired samples t-test was used. Normality was tested with the Shapiro–Wilk and in case of non-normality, a Wilcoxon signed-rank test was performed.

To satisfy the assumption of normality, the gains of admittance and reflexes were log-transformed. Sphericity was checked with Mauchly’s test and if the

assumption of sphericity was violated, a Greenhouse–Geisser correction was used (Girden, 1992). To investigate whether the admittance gain or reflex gain between the first measurement and the retest significantly differed, a 2 factor (measurement-day [2] × frequency [18]) repeated measures ANOVA was performed. Significant main effects were followed up by Bonferroni corrected pair-wise comparisons and significant interaction effects were followed up by one-way repeated measures ANOVA’s. Effects with \(P < 0.05\) were considered significant.

Because task-related modulation of admittance and reflex gain mainly occurs below the natural frequency of about 1.1 Hz (van Drunen et al., 2013), the average of the first five frequency pairs was taken to calculate the reliability of the low-frequency gains of the admittance and reflexive FRFs. To detect any significant differences between measurement-days, a paired samples t-test was performed over these low frequency gains.

To test reliability, the intraclass correlation coefficient (ICC (3,1)) was calculated according to Shout and Fleiss (1979). The ICC ranges from 0 to 1 where \(<0.40\) indicates a ‘poor’ to ‘fair’ agreement, 0.41–0.60 represents a ‘moderate’ agreement, \(0.61–0.80\) represents a ‘substantial’ agreement and \(>0.81\) represents an ‘almost perfect’ agreement (Landis and Koch, 1977).

To quantify the absolute reliability, the Standard Error of Measurement (SEM), Minimal Detectable Change (MDC) and the Limits of Agreement (LOA) were calculated. The calculation of the SEM and the MDC was performed according to Weir (2005). The LOA were calculated according to Bland and Altman (1986). Bland–Altman plots were plotted over all 18 frequency pairs to provide a visual illustration of agreement and to detect any form of bias.

All data recording, processing and system identification were done using MATLAB, version R2011a (The Mathworks, Inc., Natick, MA). All statistical analyses were conducted using IBM SPSS statistics 20.

3. Results

No significant differences were found in momentary pain and other prognostic factors measured with the questionnaires between the measurement-days in the patients. However, there was a significant difference in pain-diary scores (Table 1). The clinical important difference on a 10-point NPRS for average pain scores is 2.5 points (Farrar et al., 2010). Two patients showed a decrease of more than 2.5 points in their pain-diary scores and were therefore excluded from further analysis.

The low-back stability in both healthy subjects and patients is described by the FRF’s of admittance and reflexes (Figs. 2–4), with high coherences indicating good input–output correlations. The subject-averaged coherence exceeded the 0.05 probability level of 0.24 in both groups and conditions. Therefore, all data were used for further analysis.

No significant differences (\(P > 0.05\)) were found between measurement-day one and measurement-day two in the admittance or reflexive gains (Table 2, Figs. 2–4). Also no significant differences (\(P > 0.05\)) were found in the low frequency gains (average of frequency pairs below 1.1 Hz) of the admittance and reflexes (Table 3). The Bland Altman Plots of the admittance and reflexive gains in the resist-task (shown for healthy subjects and patients in Fig. 5) showed no forms of bias, meaning that

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Averages of the questionnaires and pain-scores in patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (± SD) measurement 1</strong></td>
<td><strong>Mean (± SD) measurement 2</strong></td>
</tr>
<tr>
<td>Momentary pain*</td>
<td>3.5 [1.8, 6.4]</td>
</tr>
<tr>
<td>Pain-diary</td>
<td>5.7 [± 1.5]</td>
</tr>
<tr>
<td>ODI</td>
<td>33.5 [± 16.1]</td>
</tr>
<tr>
<td>BBQ</td>
<td>27.1 [± 7.5]</td>
</tr>
<tr>
<td>TSK†</td>
<td>39 [33, 44]</td>
</tr>
<tr>
<td>HADS</td>
<td>10.3 [6.7]</td>
</tr>
<tr>
<td>PCS</td>
<td>17.7 [9.8]</td>
</tr>
</tbody>
</table>

df—degrees of freedom, ES—effect size. 95% CI—95% confidence interval. ODI—Oswestry Low Back Pain Disability Questionnaire; BBQ—Back Beliefs Questionnaire; TSK—Tampa Scale for Kinesiophobia; HADS—Hospital Anxiety and Depression Scale; PCS—Pain Catastrophizing Scale.

* Median (Interquartile range [IQR]).
† Z-score calculated with the Wilcoxon rank-sum test.

Significant differences between measurement-days (\(P < 0.05\)).
differences between the two measurement-days were uniformly distributed over the means. The test–retest ICC’s in patients calculated over the averaged lower frequency gains were substantial for both admittance and reflex gains (ICC_{3,1} 0.73 and 0.67 for resist-task, 0.80 and 0.70 for relax-task, see Table 4). In healthy subjects, the reliability of admittance gain in the resist-task was also substantial (ICC_{3,1} 0.66), but the ICC of the reflexive gain was only moderate (ICC_{3,1} 0.44). The SEM of the reflexive gain, however, showed lower values in healthy subjects (0.35) then in patients (0.48).

When evaluating the reliability for the 18 separate perturbation frequencies, high ICC’s were found for admittance gain over the
full range of frequencies in both healthy subjects and LBP patients (see Fig. 6, upper graph). The same consistency can be seen for the reflex gain during the resist-task in LBP patients. However, there was a decline in ICC's between 1.1 and 3.5 Hz for the reflex gain in healthy subjects during the resist-task and in patients during the relax-task and again between 13 and 15 Hz in the resist-task in healthy subjects (see Fig. 6, middle graph). Because inter-subject variability is directly related to reliability, the standard deviations (SD) of the reflex gain values between subjects were evaluated for all frequencies (de Vet et al., 2006). The SD’s for healthy subjects were lower at almost all frequencies compared LBP patients and were lowest between 1.1 and 3.5 Hz (see Fig. 6, lower graph), explaining the decrease of ICC’s in healthy subjects. The ICC’s between 1.1 and 3.5 Hz during the relax-task in patients could not be explained by inter-subject variability, suggesting that reflex gains between these frequencies, which most likely reflect velocity feedback (Schouten et al., 2008b; Pintelon and Schoukens, 2001), cannot be measured reliably during a relax-task in patients.

4. Discussion

The purpose of the present study was to determine the test-retest reliability of a method to measure trunk stability using
pseudorandom force perturbations. The reliability showed to be satisfactory for healthy subjects during a resist-task and LBP patients during both a resist-task and relax-task, with the exception of reflex gains in healthy subjects, for which reliability was only moderate. These results show that trunk stabilization can be quantified reliably, and represent a promising step towards using this method in further research in LBP patients. The small SEM’s show that within-person measurement error is moderate, which holds promise for detection of (small) differences between assessments.

The admittance gain was consistently more reliable than reflex gain in both groups across conditions, which can be explained by the inherently noisy character of EMG-signals. Also, variability in reflex sensitivity might contribute to a decreased reliability (Granata et al., 2004). To adjust for this variability and possible measurement error, averaging over more repetitions could increase the reliability of measuring reflex gains (Voglar and Sarabon, 2014), but might not be feasible for patients with LBP. Remarkably, the ICC’s (which are measures of how well subjects can be distinguished from each other) of both admittance and reflex gains were higher in patients than in healthy subjects, which might be explained by the relatively low between-subject variability and low SEM in the healthy subjects compared to the patients (Table 3) (Portney and Watkins, 2000; de Vet et al., 2006). The higher between-subject variability in patients might be due to variability of motor control impairments with LBP, as diverse changes in trunk control have been found in the literature, with evidence of decreased as well as increased trunk stiffness and reflexes (Hodges and Moseley, 2003; van Dieen et al., 2003). In line with the between-subject variability, the SEM’s were also lower in healthy subjects than in patients, which implies a lower minimal detectable change and, therefore, a higher agreement.

The present study is one of the few evaluating the reliability of measuring trunk stability (Maaswinkel et al., submitted for publication). One reliability study (interval of minimal 24 h) was performed on a method that included pseudorandom force perturbations.

### Table 3

Main effects of measurement-day on the averaged low frequency admittance and reflex gains calculated with a paired-samples t-test.

<table>
<thead>
<tr>
<th></th>
<th>Mean (± SD) measurement 1</th>
<th>Mean (± SD) measurement 2</th>
<th>df</th>
<th>t statistic</th>
<th>P value</th>
<th>ES</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resist</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admittance</td>
<td>-8.11 (± 0.23)</td>
<td>-8.02 (± 0.25)</td>
<td>12</td>
<td>-1.483</td>
<td>0.164</td>
<td>0.394</td>
<td>[-0.20, 0.04]</td>
</tr>
<tr>
<td>Reflexes</td>
<td>0.59 (± 0.47)</td>
<td>0.59 (± 0.40)</td>
<td>12</td>
<td>-0.010</td>
<td>0.992</td>
<td>0.003</td>
<td>[-0.28, 0.28]</td>
</tr>
<tr>
<td><strong>Relax</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admittance</td>
<td>-8.33 (± 0.44)</td>
<td>-8.24 (± 0.41)</td>
<td>15</td>
<td>-1.385</td>
<td>0.235</td>
<td>0.210</td>
<td>[-0.26, 0.07]</td>
</tr>
<tr>
<td>Reflexes</td>
<td>0.46 (± 0.83)</td>
<td>0.54 (± 0.53)</td>
<td>15</td>
<td>-0.533</td>
<td>0.589</td>
<td>0.096</td>
<td>[-0.39, 0.23]</td>
</tr>
</tbody>
</table>

df = degrees of freedom, ES = effect size, 95% CI = 95% Confidence interval.

Fig. 5. Bland Altman plots of the admittance gain (left) and reflexive gain (right) during the resist-task in healthy subjects (above) and LBP patients (below). The open black circles represent the differences in admittance gain or reflex gain between the two measurement-days in all subjects for all frequency points. The horizontal line represents the mean difference of the admittance and reflex gains, where the dotted lines represent the limits of agreements; in healthy subjects respectively [-0.35, 0.38] for admittance and [-1.47, 1.47] for reflexes and in patients respectively [-0.31, 0.03] for admittance and [-1.50, 1.17] for reflexes.
perturbations applied to the pelvis with a robotic platform (Reeves et al., 2014). ICC scores on position stabilizing (ICC = 0.76), flexion force stabilizing (ICC = 0.89) and extension force stabilizing (0.83) were all excellent. Hendershot et al., (2012) performed a short-term reliability study (between-day interval of 3–14 days approximately) on a method with position perturbations in healthy subjects, showing similar results on reliability to the present study. Lariviere et al., (2015) performed a medium-term reliability study (between-day interval of eight weeks) on a similar method as Hendershot et al., (2012), showing comparable reliability (Lariviere et al., 2015). A drawback of position perturbations, however, is that subjects might not be motivated enough to maximally resist the perturbation as they would during force perturbations (de Vlugt et al., 2003a, 2003b). In the present study, a distinction was made between a resist-task as a measure of the maximal stabilizing capacity and a relax-task as a more natural stabilizing task. This distinction provides information that may be relevant to identify neuromuscular control impairments in LBP patients. In an earlier conducted experiment on healthy subjects, admittance in the resist task was 61% lower (P = 0.02) and reflex gain was 71% higher (P = 0.03) than in the relax task (van Drunen et al., 2013). In the current study, task modulations in patients were less prominent, with a 3.7% lower admittance gain and 50% higher reflex gain in the resist task than in the relax task. Results suggest that patients might be less able to modulate between tasks than healthy subjects.

Three other studies on the reliability of a trunk stabilization measurement used sudden loading techniques of the trunk or upper arm on healthy subjects (Hodges et al., 2009; Voglar and Sarabon, 2014; Santos et al., 2011). Even though the ICC’s were all comparable to those in the present study, a drawback of sudden loading methods is the inability to selectively include power in the perturbation signal to allow identification of intrinsic and reflexive contribution to stabilization, which necessitates a relatively large perturbation force and a large number of repetitions, which both might not be feasible for studying trunk control in LBP patients.

There are some limitations to this study. Firstly, a relatively small number of people (13 healthy subjects and 18 patients) participated in this study. Despite the low number of subjects, reliability and agreement showed to be satisfactory. Secondly, no relax-task was performed by the healthy subjects. However, in line with the results that we found in the patients, we expect the reliability of the relax-task in healthy subjects to be comparable to the resist-task in healthy subjects. Also, there was a different time-span between the measurement-days for patients (1–2 weeks) and healthy subjects (1–3 days). One could expect reliability scores to increase when time between measurements decreases because within-subject changes that could be of influence on motor control would be less likely to take place. However, ICC’s were not higher in the group of healthy subjects who had less time between the measurements than the patients. Lastly, two patients were excluded in this study because of a decrease of > 2.5 in pain diary-scores between measurement-days. This was done to ensure similarity between both measurements. When including these patients, the ICC scores remained almost the same. Only the ICC score of the reflex-gain for the relax-task decreased from 0.70 to 0.66. Although reductions are limited, this may provide an indication of sensitivity of the measurements to change in disease severity, and therefore of the possibility to monitor disease trajectories over time. The exact relationship between changes in pain and changes in admittance and reflex gains, however, still has to be established for this method. Furthermore, the influence of known confounders such as fear of movement, illness beliefs or catastrophizing should be established to be able to interpret the relationship between disease severity and motor control.

In short, the results indicate that the test–retest reliability of admittance gain estimated using pseudorandom force perturbations is substantial in both patients and healthy subjects, while the reliability of reflex gains was substantial in patients and moderate in healthy subjects. Further research should provide insight in the impairment of motor control in LBP patients and assess if the method is responsive to changes in severity of LBP.

### Table 4
Between-day reliability of the low frequency gains.

<table>
<thead>
<tr>
<th></th>
<th>ICC3,1</th>
<th>P value</th>
<th>SEM</th>
<th>LoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resist: Healthy subjects</td>
<td>0.66</td>
<td>0.004*</td>
<td>0.13</td>
<td>[−0.46, 0.30]</td>
</tr>
<tr>
<td>Admittance</td>
<td>0.44</td>
<td>−0.01</td>
<td>0.23</td>
<td>[−0.71, 0.52]</td>
</tr>
<tr>
<td>Reflexes patients</td>
<td>0.67</td>
<td>0.002†</td>
<td>0.48</td>
<td>[−1.20, 1.05]</td>
</tr>
<tr>
<td>Relax: Healthy subjects</td>
<td>0.70</td>
<td>0.001†</td>
<td>0.48</td>
<td>[−1.30, 1.01]</td>
</tr>
<tr>
<td>Admittance</td>
<td>0.80</td>
<td>&lt; 0.001</td>
<td>0.24</td>
<td>[−0.77, 0.49]</td>
</tr>
<tr>
<td>Reflexes patients</td>
<td>0.67</td>
<td>0.002†</td>
<td>0.48</td>
<td>[−1.20, 1.05]</td>
</tr>
</tbody>
</table>

ICC3,1 - intraclass correlation coefficients [95% confidence interval]; SEM = standard error of measurement; LoA = limits of agreement.

* Significant ICC-scores

**Fig. 6.** The upper graph represents the calculated ICC’s for the admittance gain (on the Y-axis) for all frequencies [Hz] (on the X-axis). The middle graph represents the calculated ICC’s for the reflex gains for all frequencies and inter-subject SD’s for reflex gains are shown in the lower graph. The dashed lines were used to aid visibility and are not meant to imply continuous data.
Conflicts of interest statement

The authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We wish to confirm there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of the authors.

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