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Inter-Set Static Stretching does not Impair Muscle Performance and Acute Muscle Architecture

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SUMMARY

Background. Inter-set static stretching has emerged as a potential strategy for enhancing skeletal muscle adaptations. However, its impact on acute performance remains unclear.

Objective. To investigate the acute effects of inter-set static stretching on isokinetic knee extension total work and muscle swelling measured by the longitudinal muscle area variation of the vastus lateralis.

Methods. Fourteen resistance-trained men performed four sets of ten maximal repetitions of knee extension at 60°/s in an isokinetic dynamometer with 90 s of rest interval. In the inter-set stretching protocol, passive static stretching was applied until the position of maximum discomfort in knee flexion for 30s, while the other limb remained relaxed and extended as established by the control protocol. The total work performed during each repetition was summed for each set. Panoramic ultrasound images of the vastus lateralis were acquired immediately before and after the interventions and the whole longitudinal muscle area was measured. The significance level of the study was set to $P \leq 0.05$.

Results. There was a decrease in performance throughout the series in both protocols ($F = 58.334$; $P < 0.001$) and equally affected total work ($F = 1.016$; $P = 0.316$). Increases in the vastus lateralis longitudinal muscle area were observed in the control (14.8%) and experimental (20.9%) protocols, with no difference between them ($P = 0.274$).

Conclusions. Inter-set static stretching lasting 30s had no impact on knee extensor performance or muscle swelling. Coaches and practitioners can use this stretching approach without compromising exercise performance.

KEY WORDS

Extended field-of-view; isokinetic dynamometry; resistance exercise; stretching; total work.

INTRODUCTION

Stretching during the inter-set rest period is a strategy that can improve skeletal muscle adaptations and function (1, 2), such as increasing (3, 4) or no impairment (5, 6) in strength, hypertrophy (4, 5) or no impairment in hypertrophy (3, 7), and increasing in flexibility (3, 6). To understand chronic skeletal muscle morphological adaptations and optimize performance and physiological responses, inter-set stretching has also been investigated in an acute manner (8, 9). However, the inherent differences in stretching protocols and resistance exercise methodologies warrant careful consideration when interpreting these research outcomes.

There are inconsistencies in the reported acute effects of inter-set static stretching on performance-related variables.

While most studies have shown a decrease in the number of repetitions performed (10, 11), one maximal repetition test (8), and isokinetic total work (9) after inter-set stretching, two studies found no differences compared to passive rest (12, 13). The inconsistent results across studies could be attributed to variations in experimental protocols, such as differences in stretching duration, number of sets, interval time, or the outcome measures assessed. Additionally, the performance-related variables assessed may not be appropriate for investigating the effects of inter-set static stretching. The number of repetitions and maximal load tests are common metrics used in resistance exercise protocols to assess general performance. However, confounding factors, such as compensatory movements, different velocities, and underestimated or overestimated load percentages, may introduce bias in the computed result. To overcome this issue, isokinetic resistance-based protocols can provide precise and reliable measurements of the maximum torque output throughout the range of motion while controlling for angular velocity (14). The total work derived from isokinetic torque-angle output is potentially a more appropriate measure for evaluating maximal muscle performance in multiple sets when compared to the total load lifted (sets × repetitions × external load). This assumption can be attributed to the observation that both constant and variable resistance types induce accelerations and decelerations, thereby permitting maximal effort only in the concentric phase of the last repetition (15). In contrast, isokinetic resistance is characterized by maintaining a constant velocity throughout the exercise, that is, at muscle failure (15, 16). This characteristic of isokinetic resistance results in a greater amount of force being generated across all joint angles and repetitions. Consequently, it provides a comprehensive representation of the effort exerted during a maximal force task, encompassing both the concentric and eccentric phases (17).

Acute changes in muscle morphology can enhance the understanding of structural adaptations in response to a given stimulus (18). Exercise-induced hyperemia, resulting from heightened tissue metabolic activity, muscle pump action, and vasodilator mechanisms (19), begins within the first second of exercise and can persist for hours, depending on the exercise intensity (20, 21). The assumptions for adding stretching between the series of resistance exercises are stimulating hormones and higher acute levels of metabolites, resulting in greater muscle thickness as the potential superior effects of inter-set static stretching on acute metabolic stress (8, 9). Nonetheless, it should be noted that muscle thickness is usually measured only at a single site of the muscle and may not fully reflect the changes throughout the entire muscle length (22). Therefore, a two-dimensional variable, such as a longitudinal area, may be more appropriate.

In this study, we investigated the acute effects of inter-set stretching on isokinetic knee extension total work and longitudinal muscle area variation of the vastus lateralis. Specifically, we aimed to determine whether the total work and the longitudinal muscle area changed similarly in the control (passive rest) and experimental (inter-set static stretching) protocols. If inter-set stretching adversely affects knee extensor performance, we hypothesize that the total work achieved in the inter-set stretching condition will be less than the control group (9). Furthermore, since inter-set stretching increases metabolic stress (13), we anticipate greater longitudinal muscle area variation in this condition.

MATERIALS AND METHODS

Experimental approach to the problem

The study involved a data acquisition process that comprised three separate visits. Muscle-damaging eccentric contractions could lead to central fatigue through increased afferent nerve signaling of pain from sore muscles, reducing strength immediately and for the subsequent 24 hours (23). Considering that the protocol involved eccentric contractions, we determine 48 hours as the rest time between visits. The first visit was allocated to familiarizing the participants with the knee extension exercise protocol using an isokinetic dynamometer. During the subsequent two visits, participants engaged in a maximal knee extension exercise, with random assignment to either the inter-set static stretching or passive recovery protocol. Furthermore, the legs were assigned randomly, considering the preferred leg (right or left) to reduce potential bias or order effects. The study also involved the acquisition of ultrasound images of the vastus lateralis muscle before and after the isokinetic exercise. The vastus lateralis is the largest muscle of the quadriceps femoris, accounting for approximately 40% of its volume (24). Given its significant size, it is considered the most representative muscle of the knee extensor group.

Subjects

The present study recruited 14 resistance-trained men (mean ± standard deviation: age 31.1 ± 5.1 years; height 178.0 ± 7.8 cm; body mass 81.6 ± 10.7 kg) who had engaged in lower-limb exercise practices for at least one year, with a frequency of at least three sessions per week. Participants underwent screening to confirm the absence of lower limb skeletal muscle injuries or pain during daily activities before and throughout

the experimental period. All participants provided written informed consent and the study was approved by the Ethics and Research Council (HUCFF; number 92424818.6.0000.5257 – date of approval: September 23, 2018), following the latest revision of the Declaration of Helsinki.

Procedures

The isokinetic exercise was performed using a Biodex 4 System Pro isokinetic dynamometer (Biodex Medical Systems Inc.®, USA). The participants were instructed to perform five repetitions of concentric/concentric knee extension/flexion at an angular velocity of 180°/s as part of the warm-up routine protocol. The protocols were based on similar studies (6, 9, 11, 25). Before testing, all participants were instructed to perform a full range of motion during each contraction.

The intervention involved four sets of ten maximal repetitions of concentric/eccentric knee extension at 60°/s for both the inter-set static stretching and passive rest protocols. The range of motion was set at 80º (20° to 100° of knee flexion; 0 = fully extended), with a 90 s inter-set rest period. During the passive rest protocol, the participants were required to remain seated on the dynamometer seat with the thigh at rest and the knee at an angle of 80° of flexion. During the inter-set static stretching protocol, the participants remained in the prone position on a stretcher. The quadriceps femoris was passively stretched by an evaluator that flexed the participant's knee for a duration of 30s, until the maximum referred discomfort. The initial and final 30 s of the recovery interval were stipulated for the participants to return to the isokinetic seat. The participants were strongly verbally encouraged to exert the maximum possible effort during the knee extension exercises and to complete the full range of motion during each repetition.

Total work

The total work performed during each repetition and each contraction phase (*i.e.*, eccentric and concentric) was calculated by a custom Matlab script (The MathWorks Inc., Natick, Massachusetts, USA). The script integrated the joint's angular position (in radians) and the corresponding torque (in Newton meters) using trapezoidal numerical integration (26). The total work achieved in each repetition was summed to obtain the total work performed in each set. To enable the comparison of protocols conducted on different days, the data were normalized using the total work achieved in the first set of each condition as the reference value (*e.g.*, total work set 1/total work reference value × 100 = 100%).

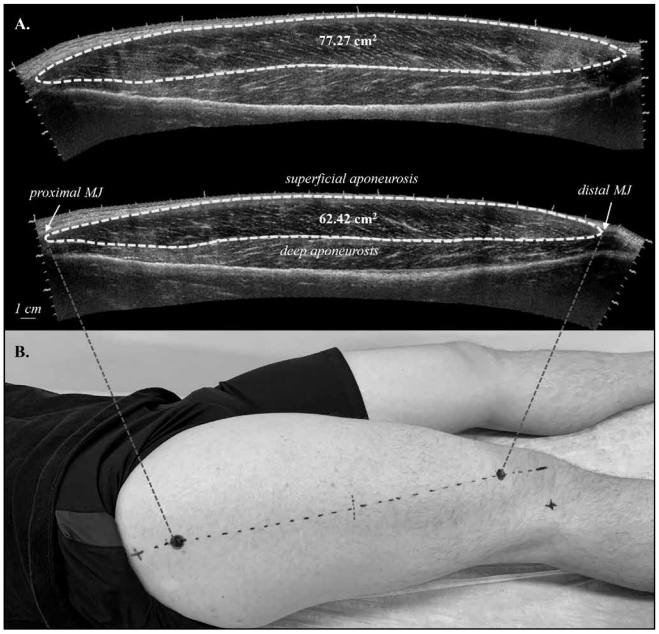


Figure 1. (A) B-mode ultrasound image acquisition and longitudinal muscle area measured pre- (bottom) and post-exercise (upper) on a participant. Deep and superficial aponeurosis and proximal and distal myotendinous junctions served as a reference for identifying the longitudinal muscle area of the vastus lateralis muscle; **(B)** The myotendinous junctions (black circles) were identified with ultrasound and the reference line (dashed line on the skin) served as a guide for the ultrasound probe to be slid. The internal region of the aponeuroses was demarcated and considered to calculate the longitudinal muscle area.

Proximal and distal MJ: proximal and distal myotendinous junctions.

Longitudinal muscle area

An experienced evaluator measured the longitudinal muscle area of the vastus lateralis muscle through ultrasound imaging using the extended field of view (panoramic) mode. The B-mode ultrasound images were acquired immediately before and after the interventions on each day. The measurement involved the following steps: 1) the participants were required to lie on the stretcher with their knee and hip extended while the hip was internally rotated; 2) the midpoint between the patella and the lateral epicondyle of the femur was identified, and a reference line was drawn from that point to the greater trochanter (dotted line, **figure 1**); 3) the proximal and distal myotendinous junctions of the vastus lateralis muscle were identified by ultrasound and marked on the skin (black circles, **figure 1**) where the reference line intersects with them; 4) the superficial and

deep aponeuroses' inner layers were meticulously circumvented. A Logic-e GE Pro ultrasound equipment (Healthcare, USA; 40 mm linear transducer, 10 MHz of excitatory frequency) was used to acquire the images of the vastus lateralis muscle. A transmission gel was applied to enhance acoustic coupling between the probe and the skin. The image quality was assessed by an experienced evaluator, who considered an image to be of good quality if both the superficial and deep aponeuroses were fully visible. The largest area delineated in the image was selected for the analysis (**figure 1**). All ultrasound images were obtained in a blinded manner using ImageJ (National Institutes of Health, USA, v.1.52), a reliable software for analysis of panoramic ultrasound of skeletal muscle (intra-rater reliability greater than 0.961) (27).

Statistical analysis

The intraclass correlation coefficient (ICC) and the coefficient of variation (CV) were used to assess the inter-day reliability of the longitudinal muscle area before the intervention and the total work performed in the first set of each protocol (baseline condition). The ICC values were calculated using the two-way mixed-effects model and absolute agreement definition (28) and interpreted by thresholds (poor: 0.00-0.39; fair: 0.40-0.59; good: 0.60-0.74; excellent: 0.75-1.00) (29). The average CV across participants was calculated for each variable and interpreted as good if CV < 5%, moderate if 5% < CV < 10%, or poor if CV > 10%. Parametric analysis was considered for inferential statistics after ensuring data normality (Shapiro-Wilk test; $p > 0.05$, for all cases) and homoscedasticity (Levene's test; $p = 0.19$). A paired sample t-test was applied to compare percentual variations in the longitudinal muscle area between control and inter-set static stretching protocols. Two-way ANOVA was applied to test the significance of inter-set static stretching on total work achieved in multiple sets of isokinetic knee extension exercise and the Bonferroni post-hoc adjustment method was used for paired comparisons whenever the main effects were verified. The significance level of the study was set to $p \leq 0.05$ and all statistical analyses were performed in R (version 4.2.2), using RStudio environment.

RESULTS

Reliability results

The results of the reliability analysis for total work and longitudinal muscle area indicate that the measures are highly reliable with consistent measurements between days. The ICC for total work was 0.856 with a 95%CI 0.565-0.953, indicating excellent reliability between measurements. The CV was 6.42%, suggesting moderate variability between the measurements. Similarly, the ICC for the longitudinal muscle area was 0.737 with 95%CI 0.303-0.911, indicating excellent reliability between measurements. The CV for the longitudinal muscle area was 6.94%, indicating moderate variability between measurements.

Changes in total work

The absolute mean ± standard deviation of total work performed throughout sets 1,2,3, and 4, were respectively 3,878.25 ± 885.09 J; 3,528.33 ± 772.06 J; 3,062.86 ± 700.35 J; 2,764.78 ± 648.06 J for the control protocol, and 3,647.8 ± 731.56 J; 3,368.907 ± 719.12 J; 3,008.01 ± 622.38 J; 2,696.873 ± 510.18 J for the inter-set static stretching protocol. As observed in **figure 2**, there was a main effect for set on the delta total work ($F = 58.334$; $p < 0.001$), revealing a decrease in performance throughout sets in both protocols. However, the effect was not significant for the "protocol X set" interaction ($F = 0.176$; $p = 0.913$), and the main effect for "protocol" was not significant ($F = 1.016$; $p = 0.316$), indicating that both protocols affect equally the muscle performance.

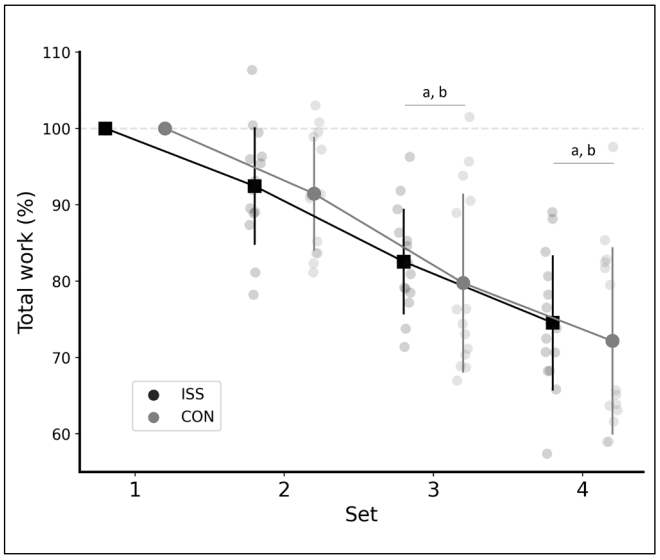


Figure 2. Normalized total work (set one) and the relative decrease in sets two to four, with respective individual data for both inter-set static stretching and control protocols.
ISS: inter-set stretching protocol; CON: control protocol; a. significant difference from set 1; b. significant difference from set 2.

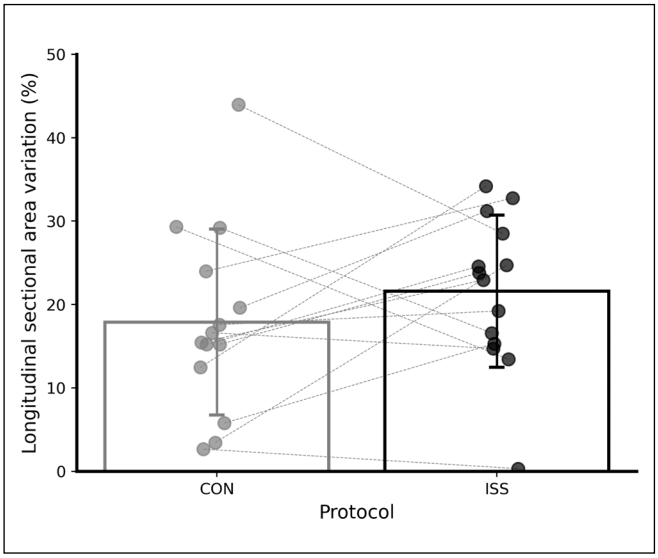


Figure 3. Longitudinal muscle area variation pre and post exercise, with respective individual data for both inter-set static stretching and control protocols.
ISS: inter-set stretching protocol; CON: control protocol.

Changes in the longitudinal muscle area

The absolute mean ± standard deviation of the longitudinal muscle area measured pre- and post-exercise, was respectively 64.14 ± 9.63 cm^2; 73.64 ± 7.2 cm^2 for the control protocol (14.81% increase), and 59.11 ± 9.65 cm^2; 71.49 ± 9.87 cm^2 for the inter-set static stretching protocol (20.94% increase). The grouped data revealed that both protocols were able to increase the vastus lateralis longitudinal muscle area. However, as shown in **figure 3**, there was no significant difference between the protocols when considering delta values ($p = 0.274$).

DISCUSSION

The present study aimed to investigate the acute effects of inter-set stretching on the total work of the knee extensors and vastus lateralis longitudinal muscle area variation. Our findings indicate that there were no significant differences in total work between sets (2-1, 3-1, and 4-1) when the inter-set stretching was compared with the passive rest protocol. Additionally, the inter-set stretch did not cause any significant changes in the longitudinal muscle area compared to the control response, suggesting that there were no further increases in blood flow redistribution of the stretched limb. The inter-set static stretching method is frequently employed as a time-efficient approach to target muscle hypertrophy (30). In our study, we utilized a rest interval that allowed sufficient time for applying the stretching protocol. It was expected that the non-stretched limb would experience a decrease in performance, as 90 s is insufficient for the recovery of adenosine triphosphate and phosphocreatine, the clearance of fatigue-causing intramuscular metabolites, and the restoration of force production (31, 32), as confirmed in **figure 2**. Despite these concerns, inter-set static stretching did not accentuate fatigue (*i.e.*, a decrease in total work) in our study. Existing systematic reviews indicate that significant performance losses occur when stretching durations exceed 60 s, while only trivial effects are observed for durations below 60 s (33, 34). This suggests a dose-response relationship between stretching volume and performance decrement.

The inter-set static stretching method involves subdividing longer stretch durations into a series of shorter stretch durations.

In our study, each stretching series of the experimental protocol consisted of 30 s of static stretching and 60 s of passive rest. In clinical practice, stretching is commonly performed for ≥ 60 s, although it has a deleterious effect on performance (33, 34). Even if the total stretching volume (*i.e.*, 90 s) exceeds the threshold of 60 s, our findings indicate that longer static stretching organized into multiple shorter sets performed between sets does not negatively impact performance. Previous studies observed a decrease in performance following inter-set stretching (6, 8-10), while others found no significant differences compared to passive rest (12, 13), probably due to different stretching protocols applied. For instance, some studies employed passive static stretching (6, 8, 13), while others used active static stretching (6, 10-12) ranging from 25 s to 1 minute in each stretches. Additionally, the number of stretching sets varied from three to seven, resulting in varying total stretching volumes throughout the series. The performance-related variables, assessed through the number of repetitions and maximal load tests, are common metrics used in resistance exercise protocols to evaluate general performance. However, these variables are subject to confounding factors, including compensatory movements, varying velocities, and inaccurate estimations of load percentages (35).

To assess muscle fatigue in a controlled manner, we conducted our study using an isokinetic dynamometer, measuring the total work variable for both concentric and eccentric contractions in all series (36). Only two studies (9, 12) utilized the isokinetic dynamometer, both focusing on maximal leg extension performance. Cometti *et al.* (12) study involved six sets of intra-set stretching lasting 1

minute each, totaling 5 minutes of stretching volume. The velocity used was 120°/s, and the maximal voluntary torque was the performance variable, with a 3-minute rest interval between sets. Padilha *et al.* (9) employed a velocity of 60°/s and assessed total work to evaluate participant performance, with a stretching duration of 25 s, similar to ours (30 s). However, they used seven sets of 10 repetitions, resulting in a total stretching volume of 150 s – 60 s longer than protocol of the present study. After the seven sets, the total work of the experimental group decreased. In our study, the inter-set static stretching doesn't influence the performance across the four series. It is unclear if there were significant differences between sets, as the performance between sets was not evaluated in Padilha's study (9). A greater number of sets may lead to group differences in our study, although figure 2 does not demonstrate such a trend. Resting time intervals can also play a role in that scenario. In our study, longer resting interval pos stretching (60 s) compared to 15 s from Padilha *et al.* (9) could have been crucial to performance maintenance. Based on these findings, the inclusion of shorter stretch durations combined with passive recovery intervals may have contributed to the absence of disparity between the protocols utilized in this study.

Chronically, the studies show intra-set stretching leads to superior strength and flexibility gains compared to passive rest between sets (4, 6, 9, 37); and induces positive muscle adaptations, including hypertrophy, without increasing total exercise time (4, 5). The authors suggest that greater time under tension and muscle blood flow restriction for the inter-set stretching group may explain differences between hypertrophic adaptations (4, 5). Acutely, greater time under tension and muscle blood flow restriction can affect metabolic, hormonal, and physiological responses, such as an increase in leukocytes and lactate, reflecting muscle swelling increases (13). In addition, redirection of blood flow to the exercised muscle group may reflect muscle swelling and has been assessed through changes in muscle thickness, showing an increase up to 15 min after resistance exercise (38). In this sense, Camargo *et al.* (8) found a decrease in muscle thickness in the inter-set stretching group and explained the results by the decrease in the number of repetitions.

It is essential to consider that muscle thickness represents a measurement of a single muscle length, and thus, this singular location may not fully reflect the behavior of the entire muscle (22). Utilizing a measure of muscle architecture capable of assessing muscle swelling across the entire muscle length may offer a more comprehensive perspective (**figure 1**). In our study, longitudinal muscle area did not exhibit significant differences between groups, consistent with findings reported by Padilha *et al.* (9), who also assessed muscle thickness. Discrepancies in results may be attributed to variations in the muscle group assessed (8) and the number of sets employed (9).

Considering that in our study there were no differences in total work did between protocols, we suggest that changes in muscle swelling could be associated with muscle work during muscle contraction. One potential explanation for this hypothesis is related to the spatial limitation of muscle tissue. Muscles are isovolumetric structures and surrounded by a layer of connective tissue, which imposes limitation on blood flow (39). As a result, the effect of stretching may be insignificant compared to the effect of muscle contractions performed during exercise if they already provoke an increase close to the maximum. To explore this premise, future studies could consider acquiring images between sets or monitoring the subacute inflammatory process in the days following the intervention. Measuring the longitudinal muscle area between the series is intuitive since there was an assessment of the total work in the four series and in this way, the edema behavior could be associated for each of the series. However, the panoramic ultrasound images require time to be acquired and are a limitation of the technique, as the interval would be too long for the present investigation. Another limitation of our study was the absence of edema assessment and metabolic responses in the days following the intervention. Stretching can acutely and subacutely activate mechanisms such as the inflammatory process, and insulin-like and myogenic growth factors (1). Evaluating these parameters in future studies could provide valuable insights into inter-set stretching, as inflammation-induced muscle edema is associated with morphological adaptations (30). Despite the limitations, it is important to highlight that this study seems to be the first to evaluate 1) the total work performed between sets in inter-set stretching and control groups, and 2) the response of muscle swelling throughout the length of the muscle.

CONCLUSIONS

The findings of this investigation indicate that integrating 30-s intra-set quadriceps stretching during maximal leg extension exercise does not impact the total work over four sets, nor does it increase muscle swelling in physically active men. This suggests that coaches and practitioners can benefit from this time-efficient approach for the targeted muscle group without compromising exercise performance. Furthermore, we advise maintaining a rest interval of at least 60 s, in addition to the stretching duration, to preserve the training volume. However, caution should be

exercised when extrapolating these results to other exercises and muscle groups.

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None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

GHH: conceptualization, methodology, data collection, writing - original draft. JCSA: data evaluation, formal analysis, writing - review & editing. BFL: data curation, writing - review & editing. LFO: supervision, writing - review & editing, project administration. TTM: conceptualization, methodology, supervision, writing - review & editing, project administration.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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