

## The effects of eccentric exercise on passive hamstring muscle stiffness: Comparison of shear-wave elastography and passive knee torque outcomes

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### Abstract

The aim of our study was to assess eccentric-exercise-induced changes in passive knee joint torque, passive knee joint stiffness and shear modulus at of the hamstring muscles. We hypothesized that eccentric exercise would elicit an increase in all outcomes. Fourteen healthy volunteers (age =  $25.5 \pm 4.7$  years) performed eccentric exercise protocol. Before and after 0h, 1h, 24h and 48h, we measured the shear modulus of hamstring muscles using shear-wave elastography and passive knee joint stiffness on isokinetic dynamometer. After eccentric exercise, the shear modulus of biceps femoris increased after 0h ( $22.4 \pm 34.1$  %;  $p = 0.021$ ) and for semitendinosus after 0h ( $14.5 \pm 4.9$  %), 1h ( $16.2 \pm 6.5$  %) and 24h ( $16.6 \pm 8.3$  %) ( $p = 0.005$ - $0.015$ ). There were no changes for semimembranosus and no changes in passive knee joint moment measures. There were also no correlations between the two methods. Eccentric exercise increased shear modulus of hamstring muscles, while passive joint torque was not affected. This suggests that shear-wave elastography could be more sensitive than torque measures to intra-muscular changes induced by eccentric exercise.

**Key Words:** Shear modulus; muscle damage; muscle stiffness; eccentric exercise; passive stiffness.

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Muscle stiffness is an important trait, significantly affecting human movement.<sup>1</sup> For instance, gastrocnemius muscle stiffness was reported to be associated with drop jump performance.<sup>2</sup> Moreover, changes in muscle stiffness are an important marker of neuromuscular diseases, such as cerebral palsy<sup>3</sup> and Parkinson's disease.<sup>4</sup> Muscle stiffness is also affected by various exercise,<sup>1,5</sup> muscle damage<sup>6</sup> and menstrual cycle.<sup>7</sup> Therefore, there are several areas of application for assessment of muscle stiffness, with several different methods being used in the literature, including myotonometry,<sup>8,9</sup> passive joint torque measurements,<sup>10-13</sup> and quantification of shear modulus through ultrasound-based shear-wave elastography.<sup>12-14</sup> However, it is not entirely clear if these methods can be used interchangeably. Studies investigating acute effects of different exercise modalities on different measures of muscle stiffness are abundant. Static stretching has been shown to decrease both passive joint torque and muscle

shear modulus, and the magnitude of the effect seem to be dependent on factors such as intensity and volume of the stretching.<sup>15,16</sup> However, while dynamic stretching also decreases passive joint torque,<sup>17</sup> it seems to increase the muscle shear modulus.<sup>14</sup> While further studies are needed to clarify this difference, caution should be taken when comparing the assessments of both passive joint torque and muscle shear modulus after stretching interventions. Regarding the chronic effects, static stretching has been shown to decrease both passive joint torque<sup>11</sup> and shear modulus.<sup>18</sup> On the other hand, one study conducted on gastrocnemius muscles reported decreases in shear modulus as well as passive joint torque after static stretching,<sup>19</sup> however, the former was only decreased at the end of the range of motion. Another interesting avenue for the comparison of methods to assess muscle stiffness is studying the changes induced by eccentric exercise. Previous studies have documented an increase in shear modulus and

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passive joint torque after eccentric exercise.<sup>20-22</sup> One study explored the changes in shear modulus and muscle stiffness as assessed by myotonometry, and while both methods indicated an increased muscle stiffness, there were no correlations between changes in both methods.<sup>9</sup> This indicates that the methods should probably not be used interchangeably. While we are not aware of any studies that assessed changes in shear modulus alongside changes in passive joint torque after eccentric exercise, a recent study used both methods to assess changes induced by dermal suction therapy.<sup>23</sup> The authors reported statistically significant increases in shear modulus, and no changes in passive joint stiffness,<sup>23</sup> again pointing to a need for caution when using different methods to assess muscle stiffness.

Considering the previous evidence outlined above, the aim of our study was to assess eccentric-exercise-induced changes in passive knee joint torque, passive knee joint stiffness and shear modulus of the hamstring muscles. We hypothesized that eccentric exercise will elicit an increase in all outcomes.<sup>9,22</sup> We also hypothesized that the changes in each of the methods will be in trivial or small correlations.<sup>23</sup> The findings of this study could be important for clinical practice and future research on muscle characteristics. Namely, we will reveal if (and to what extent) the shear modulus and passive joint torque measurement can be used interchangeably to infer changes in muscle stiffness.

### Materials and Methods

#### Participants

Previous studies have reported large or very large effects (effect size  $\sim 1.0$ ) of eccentric exercise on muscle stiffness<sup>20,21</sup> and our a priori analysis ( $\alpha = 0.05$ ; power = 90 %) indicated that 11 participants were

sufficient for the study. Fourteen healthy volunteers (age= $25.5 \pm 4.7$  years, body mass= $69.3 \pm 13.8$  kg, height= $171.6 \pm 9.5$  cm) were included in the study sample (7 males, 7 females). Inclusion criteria were regular engagement in physical activity (at least 3 hours per week) and age between 20 and 40 years. Participants were excluded if they reported any musculoskeletal injuries or pain in the past six months, were currently competing in any sport or had serious cardiovascular or systemic disease. Upon arrival participants gave written consent and were informed about the study purpose and possible risks of participation. The study protocol was approved by the National Medical Ethics Committee (approval number: 0120-557/2017/4) and was conducted according to the Helsinki Declaration.

#### Study design

This was a single-group repeated measures study. Measurements of passive muscle stiffness and passive isokinetic torque were assessed in three sessions at five different time points: at baseline, immediately (Post0h) and 60 minutes (Post1h) following eccentric training (session one), at 24 h (session two, Post24h) and 48 h (session 3, Post48h) after eccentric exercise protocol. During the first session, participants completed a 10 minute aerobic warm-up on a cycloergometer prior to eccentric training. The leg which was included in the experiment was randomized for each participant. At the beginning of session two and three participants reported the level of perceived muscle soreness on a scale of 0–10 (0 referring to no pain and 10 to worst pain imaginable).

#### Shear-wave elastography

Shear modulus of hamstring muscles (biceps femoris - BF, semitendinosus - ST and semimembranosus - SM)



**Fig 1.** Participant positioning for shear-wave elastography measurements.

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was measured using shear-wave elastography with an ultrasound device (Resona 7, Mindray, Shenzhen, China). The sound touch quantification mode was used, since it enables direct quantification of shear modulus values. The ultrasound system was set to musculoskeletal SWE mode, which assumes the tissue density of 1000 kg/m<sup>3</sup>. Middle-sized linear probe (Model L11-3U, Mindray, Shenzhen, China) was used. We applied a generous amount of water-soluble, hypoallergenic ultrasound gel (AquaUltra Basic, Ultrigel, Budapest, Hungary) to the probe. The region of interest was set at 1×1 cm. The depth of the region of interest was chosen for each participant individually in order to ensure that only muscle tissue was captured. The value for depth was noted and kept constant throughout the sessions.

Participants were positioned in prone lying on the edge of a therapeutic table, with the hip and knee at 60° and 30° flexion, respectively (Figure 1). It was proposed that positioning the muscles in a prestretched position can improve the reliability of measurements.<sup>24</sup> At each time point participants laid in the aforementioned position for 5 minutes prior to measurement to account for possible stretch-relaxation effects. Additionally, room temperature was held constant at 20° due to possible effects on muscle stiffness. Prior to baseline measurements the exact location and orientation of the probe was marked with a permanent marker. The probe was positioned at approximately half of the distance between the ischial tuberosity and popliteal fossa and oriented parallel to the muscle fibers. It was rotated until both the fascicles and superficial fascia were uninterrupted on the image. During one measurement eight consecutive scans were performed, and the median value of two measurements at each time point was considered for further analysis. In contrast with previous studies, we have included the median value since it is less sensitive to outliers that can occur during measurements.

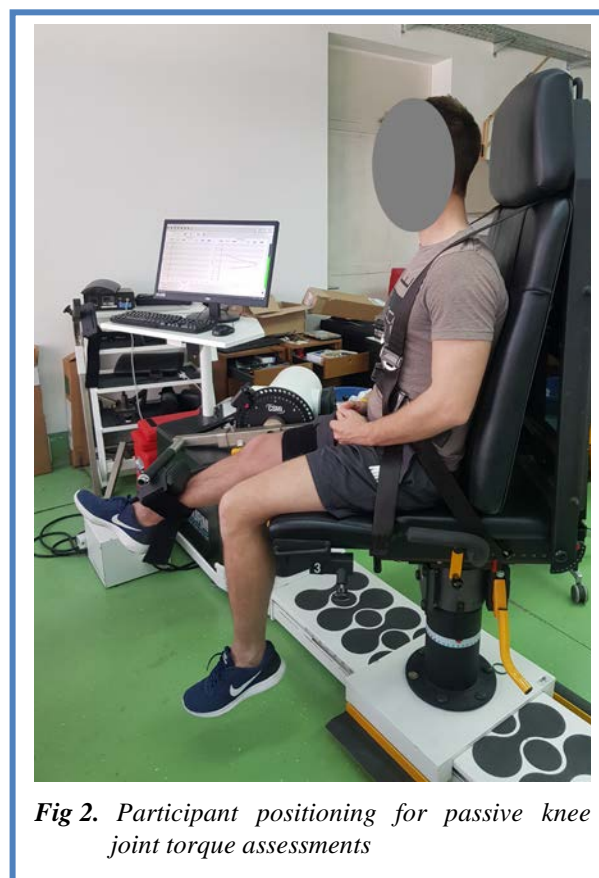
### *Passive joint torque and stiffness*

Participants were positioned on an isokinetic dynamometer device (Humac Norm, Computer Sports Medicine Inc., Massachusetts, US) in seated position (hips in 90° of flexion and in neutral position in horizontal and frontal plane). Device harness stabilized the upper body and straps were used to fixate the pelvis and the distal thigh just above the knee joint.<sup>17</sup> The axis of rotation of the dynamometer was aligned with the lateral epicondyle of the knee and lever arm was fixated with the strap just above the malleoli (Figure 2). Limits for range of motion and correction for the weight of the measured segment were set prior to measurements. Measurements were performed at slow angle velocity of 5°/s to avoid reflexive or voluntary protective muscle contractions. Participants were instructed to stay relaxed throughout the measurement. Passive isokinetic torque was measured in the range from 70° to 0° of knee flexion. One set of three cycles

was performed as a familiarization with the procedure prior to each measurement. One set consisting of five consecutive cycles of passive movements were measured and the middle three cycles were used in further analysis. We chose to analyze the mean torque in 0-5° and 0-35° and apparent passive joint stiffness in 35-0° of knee flexion range of motion (0° = full knee extension). Namely, the first half of the range of motion (70-35° of knee flexion) was often contaminated with mechanical measurement artefacts, related to the initial acceleration of the limb-lever system. Passive joint stiffness was expressed as a slope of the angle-torque curve the joint moment-angle curve from 35° to 0° of knee flexion. This was calculated by fitting a fourth-order polynomial through the data from 35° to 0° of knee flexion.<sup>13</sup>

### *Eccentric exercise protocol*

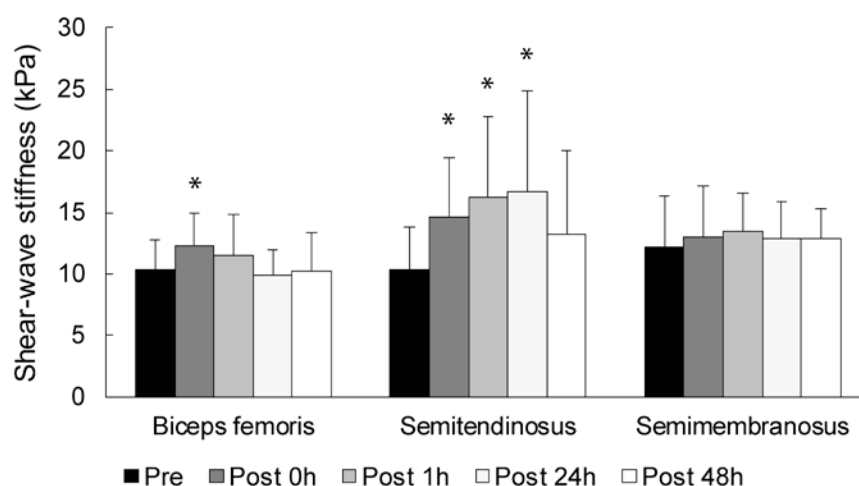
Participants completed a protocol of eccentric training, which included maximal eccentric knee extensions on an isokinetic dynamometer and Nordic hamstring exercise. For the former, we used the same set-up as described previously. Isokinetic eccentric training consisted of 3 sets of 10 repetitions, interspersed with two minutes of rest. For the eccentric part of the movement, the participants were instructed to resist the machine movement by attempting to pull the heel toward their buttock with maximal exertion. For the concentric part they were instructed to perform the same



**Fig 2.** Participant positioning for passive knee joint torque assessments

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**Fig 3.** Changes in shear-wave muscle stiffness (i.e., shear modulus) after the eccentric exercise. \*denotes statistically significant differences from the pre-exercise measures

movement with minimal effort. Following the isokinetic protocol, participants performed 3 sets of 6 repetitions of the Nordic hamstring exercise. Two minutes of rest were implemented between sets. Participants were kneeling on a foam pad. The researcher stabilized the participant's shins using his bodyweight. The participants were asked to lower their body toward the ground in a slow and controlled manner. Participants were allowed to perform the exercise with the hip in a flexed position (not more than 45°) of to achieve controlled movement over a larger range of motion.

### Statistical analysis

Statistical analyses were done with SPSS (version 25.0, SPSS Inc., Chicago, IL, USA). Descriptive statistics are reported as mean  $\pm$  standard deviation. The normality of the data distribution was verified with Shapiro-Wilk tests. The effect of eccentric exercise was assessed with one-way repeated measures analysis of variance (ANOVA), with 5 time points considered as within-subject factors. We used Bonferonni corrected post-hoc t-tests to assess the differences among individual time points. Effect sizes were expressed as partial eta-squared ( $\eta^2$ ) and interpreted as trivial ( $<0.01$ ), small (0.01-0.06), medium (0.06-0.14) and large ( $> 0.14$ )<sup>25</sup>. Using Pearson product-moment correlations, we assessed: a) the correlations between shear-wave and passive torque outcomes at baseline and b) the correlations between relative changes observed in each outcome. The threshold for statistical significance was set at  $\alpha < 0.05$

## Results

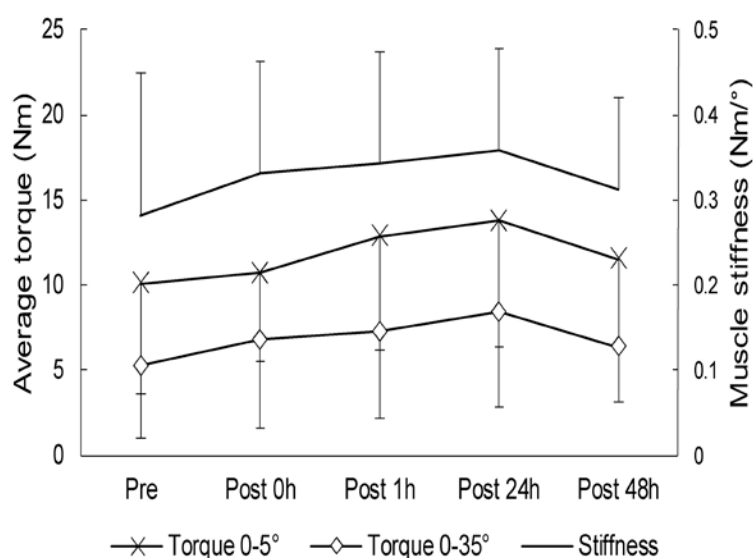
### Effect of eccentric exercise

Eccentric training resulted in the occurrence of muscle soreness (Post24h:  $4.36 \pm 1.55$ , Post48h:  $6.46 \pm 1.50$ ). The ANOVA revealed a statistically significant large

effect of eccentric exercise on BF shear modulus ( $F = 5.65$ ;  $p = 0.009$ ;  $\eta^2 = 0.22$ ). Post-hoc tests revealed that compared to baseline, the values were statistically significantly elevated only at Post0h ( $22.4 \pm 34.1\%$ ;  $p = 0.021$ ), but not at the other time points. For the ST, ANOVA also revealed a statistically significant large effect of eccentric exercise ( $F = 8.51$ ;  $p = 0.001$ ;  $\eta^2 = 0.31$ ). Compared to baseline, the values were statistically significantly elevated at Post0h ( $14.5 \pm 4.9\%$ ), Post1h ( $16.2 \pm 6.5\%$ ) and Post24h ( $16.6 \pm 8.3\%$ ) ( $p = 0.005-0.015$ ). For the SM muscle, the effect of eccentric exercise was not statistically significant ( $F = 0.65$ ;  $p = 0.696$ ). The results are displayed on Figure 3. The results regarding average torque in 0-5° and 0-35°, as well as passive stiffness (joint moment-angle curve slope), are displayed in Figure 4. Unlike with the shear-wave based outcomes, there were no statistically significant effects of eccentric exercise ( $F = 1.16-2.10$ ;  $p = 0.096-0.340$ ), although the effect size for passive joint stiffness (joint moment-angle slope) was rather large ( $\eta^2 = 0.16$ ).

### Correlations

At baseline, there was a statistically significant moderate correlation between BF and ST shear moduli ( $r = 0.57$ ;  $p = 0.032$ ), as well as among all three outcomes measured by passive joint motion (i.e., average torque in 0-5° and 0-35° range of motion and joint stiffness;  $r = 0.74-0.94$ ). Changes in BF and ST moduli at Post0 were also in statistically significant moderate correlation ( $r = 0.54$ ,  $p = 0.047$ ). When changes to Post1h were analyzed, there was a statistically significant moderate correlation between BF and ST ( $r = 0.55$ ;  $p = 0.049$ ), BF and SM ( $r = 0.82$ ;  $p < 0.001$ ) as well as SM and ST ( $r = 0.59$ ;  $p = 0.027$ ) shear moduli. Moreover, changes Post1h in both torque measures (0-5° and 0-35°) were in statistically



**Fig 4.** Changes in average torque in 0-5° and 0-35° range of motion (left axis, bottom two lines) and muscle stiffness (right axis, upper line) after the eccentric exercise

significant high correlation ( $r = 0.72$ ;  $p = 0.006$ ), which was also the case Post24h ( $r = 0.94$ ;  $p < 0.001$ ). Changes at Post48h for all three outcomes measured by passive joint motion (i.e., average torque in 0-5° and 0-35° range of motion and joint stiffness) were also inter-related, with statistically significant moderate to high correlations ( $r = 0.62-0.82$ ;  $p = 0.001-0.024$ ). Changes in shear-wave outcomes were not inter-related at Post24h or Post48h. There were no correlations between changes in shear wave outcomes and changes in passive joint torque or stiffness measurement at any time point.

## Discussion

The aim of this study was to assess the effects of one bout of eccentric exercise on passive joint torque and shear modulus of the hamstring muscles (BF, ST and SM). Only shear modulus was increased after the eccentric exercise (BT and ST, but not SM), whereas passive joint torque and stiffness remained unaffected. Therefore, our first hypothesis was only partially supported. Moreover, while there were some correlations between different variables obtained by the same method, there were no correlations between changes in shear modulus and changes in passive joint torque or stiffness. Therefore, we confirmed the second hypothesis by demonstrating that changes in shear modulus after eccentric exercise do not parallel the changes in passive joint torque measurements.

An increase in shear modulus after eccentric exercise is in accordance to previous studies.<sup>20,21</sup> These increases were muscle-specific, as SM showed no changes. Although muscle activity was not formally measured in this study, previous research findings indicate lower activation of SM during Nordic hamstring exercise,

which could partially explain the observed muscle-specific changes in our study.<sup>25-26</sup> The exact mechanisms behind the increases in muscle stiffness are still unclear. Several possible factors, such as swelling,<sup>27</sup> injury-related contracture,<sup>22</sup> and cytoskeletal damage,<sup>10</sup> have been suggested. An interesting finding of our study is that BF passive stiffness was not higher at Post24h and Post48h compared to baseline, despite the presence of muscle soreness (Post24h:  $4.36 \pm 1.55$ , Post48h:  $6.46 \pm 1.50$ ). While an increase in muscle stiffness after eccentric exercise has also been documented using myotonometry,<sup>10</sup> we observed no effect of eccentric exercise on passive joint torque. Moreover, we found no correlations between shear modulus and passive joint torque outcomes. A previous study conducted on a trapezius muscle reported no correlation between changes in shear modulus and changes in muscles stiffness as assessed with myotonometry.<sup>9</sup> Although the intervention in their study was not eccentric exercise, but dermal suction therapy, their findings (together with our results) suggest limited agreement across different methods of assessing muscle stiffness.

It is also possible that the correlations between passive torque measures and passive muscle stiffness could be angle-specific. However, shear modulus was measured only at one muscle length. It has been shown that shear modulus increases with muscle length<sup>28</sup> and it could be that length-specific shear modulus or even the slope of the relationship between muscle length (using joint angle as a proxy) and shear modulus would reveal different effects of eccentric exercise and different relationships with passive torque measurements. In our study, average torque was calculated either over a higher range of motion (35°) or at terminal knee

extension. Future studies should include passive torque measures at the same angles as shear-wave elastography measurements. Altogether, our results suggest that a) shear-wave elastography seems to be more sensitive (compared to passive joint torque measurements) to changes within the muscle induced by eccentric exercise and b) even if passive joint torque measurements are able to detect changes in muscle stiffness (be it as a result of eccentric exercise or some other intervention), the outcomes may not be used interchangeably with shear modulus (at least as assessed in this study). Although both shear-wave elastography and passive joint torque measurements are purportedly assessing muscle stiffness, there are certain differences between the methods that could explain our results. In shear-wave elastography, shear modulus is calculated from the assumed tissue density (i.e., 1000 kg/m<sup>3</sup> for muscle tissue) and the speed of the propagation of shear waves through the tissue.<sup>29</sup> In an ex vivo study,<sup>30</sup> it was shown that shear modulus values were closely related ( $R^2 = 0.93$ ) to muscle tension assessed directly through tensile testing. However, when the ultrasound probe was not aligned parallel to the orientation of the muscle fibers, the relationship was significantly weaker ( $R^2 = 0.12-0.24$ ). Thus, an important limitation of shear-wave elastography is its sensitivity to probe positioning. On the other hand, the main limitation of passive joint torque measurements is the fact that contribution of different tissues (muscle, tendon, connective tissues) is challenging to determine.<sup>31</sup> The measures of passive joint torque in the present study tended to increase after eccentric exercise that could become statistically significant if we used a larger sample size. These small changes could reflect the underlying changes in muscle stiffness, which are blunted by the contributions from other tissues. This study does not come without limitations. Our results should not be generalized to other populations such as athletes, older adults or patients with neuromuscular diseases. As mentioned, larger sample size would allow us to draw firmer conclusions regarding the changes in passive joint torque outcomes. Moreover, only one site was measured with shear-wave elastography on each muscle, neglecting potential region-specific changes in shear modulus. Although a very slow velocity of angular rotation was used for passive knee joint torque assessments, we cannot assure that no muscle activity was present at all times.

In conclusion, eccentric exercise increased shear modulus of hamstring muscles after eccentric exercise, while passive joint torque was not affected. This suggests that shear-wave elastography could be more sensitive to intra-muscular changes, induced by eccentric exercise. Additionally, the lack of correlations between shear-wave elastography and passive joint torque assessments is a further reason to avoid using the two methods interchangeably. Future research should consider to compare the two methods with applying

different interventions to induce changes in muscle stiffness.

### List of acronyms

BF – biceps femoris;  
ST – semitendinosus  
SM – semimembranosus  
ANOVA – analysis of variance

### Contributions of Authors

MV, RV and NS conceptualized the study idea. MV and RV performed the measurements. ZK performed the statistical analysis. MV, RV and ZK wrote the manuscript. All the authors reviewed the manuscript and all the authors have approved the edited final typescript

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### Conflict of Interest

The authors have no conflicts of interest to declare..

### Ethical Publication Statements

We confirm that we have read the journal's position on ethical issues involved in publication and affirm that this report is consistent with those guidelines.

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