



# Brief Report: Improving Social Outcomes for Students with Autism at Recess Through Peer-Mediated Pivotal Response Training

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## Abstract

Many students with Autism Spectrum Disorder (ASD) struggle to appropriately interact and play with their peers at recess. In this pilot feasibility study, we tested the efficacy of practitioner-implemented, peer-mediated Pivotal Response Training (PRT) with 11 elementary and middle school students with ASD. Participants were randomly assigned to a treatment or control group. We measured outcomes at multiple time points, and analyzed data using multi-level modeling with time nested within student. We demonstrated large and statistically significant increases in peer interaction ( $d = 1.13$ ). Appropriate play with peers also increased substantially ( $d = 0.89$ ). Practitioners and students provided positive feedback. These findings suggest school staff can feasibly facilitate peer-implemented PRT that improves social outcomes for students with ASD at recess.

**Keywords** Autism · Pivotal response training · Peer-mediated intervention · Inclusion · Peer interactions · Peer play

## Introduction

Two of the defining characteristics of Autism Spectrum Disorder (ASD) are deficits in social communication and interaction, and restricted and/or repetitive patterns of behavior, interests, or activities (APA 2013). These characteristics are especially evident on the playground, where many students with ASD struggle to appropriately interact with peers, and sometimes engage in stereotypic behavior that is incompatible with typical play (Anderson et al. 2004).

Researchers have investigated three different categories of intervention aimed at improving social outcomes for students with ASD at recess. These include student-focused, context-focused, and peer-focused approaches. Student-focused interventions involve direct instruction on social skills, such as a social skills groups in the classroom paired with self-monitoring during recess (Radley et al. 2014). Context-focused interventions involve changing equipment or activities to increase engagement in the activity or with peers. For example, Koegel et al. (2014) trained

paraprofessionals to modify recess games to incorporate the special interests of students with ASD. Peer-focused interventions involve training and prompting peers to use strategies that promote positive play and interaction with students with ASD. For example, Owen-DeSchryver et al. (2008) led peers in a guided discussion of how they could interact with students with ASD at recess.

In this paper, we focus on one specific peer-mediated approach: peer-implemented pivotal response training (PRT). PRT is a naturalistic intervention that focuses on motivation, responsivity to multiple cues, self-management, and social initiations (Pierce and Shreibman 1997). PRT has been identified as an evidence-based practice for students with ASD (Wong et al. 2015), and has been effectively implemented by typically developing peers in inclusive educational settings (e.g., Pierce and Shreibman 1997). Given its emphasis on loose training and generalization, PRT might be especially well suited to unstructured settings such as recess. One research group has piloted peer-implemented PRT at recess. Harper et al. (2008) measured the impact of peer-implemented PRT on the number social initiations and turn-taking exchanges of two elementary-aged boys with ASD at recess. They found that peers were able to successfully implement PRT strategies, one of the two students demonstrated a clear improvement in social initiations, and both students increased their turn-taking exchanges.

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Harper et al. (2008) made a valuable contribution to the literature, but there are limitations to their study that highlight the need for further research. These include a small sample size ( $n = 2$ ) that limits generalization of findings, mixed effects, and measures that only captured limited aspects of play at recess (i.e., initiations and turn-taking). In addition, the research team implemented the intervention themselves, making it unclear if school staff could feasibly facilitate peer-implemented PRT at recess.

In the present study, we aimed to address those limitations. Specifically, we implemented a pilot feasibility study with 11 participants. To improve generalization of findings, we measured multiple types of interactions and categories of play, and we trained school staff who were already supervising recess to facilitate the intervention. We addressed the following research questions: What are the effects of practitioner-facilitated peer-implemented PRT on (a) peer interactions and (b) the quality of their play for students with ASD at recess? We hypothesized that this intervention would increase the number of peer interactions and increase the time students with ASD engaged in appropriate play with peers. In addition, we surveyed students with ASD, their peers, and school staff to better understand their perceptions of the intervention and its effects.

## Method

### Participants

We recruited 11 students with an educational diagnosis of ASD who were not frequently interacting with peers at recess (per teacher report), 19 of their peers who did not have developmental disabilities and shared the same recess (i.e., 3–4 peers per student with ASD in the treatment group), and 11 adults who were already supervising recess and were willing to implement the intervention. Special education teachers nominated students with ASD who met inclusion criteria. All students attended elementary or middle schools in suburban school districts in a Midwestern state. We provided \$50 honorariums to adults who consented, regardless of whether they were assigned to the experimental condition. Informed consent was obtained from all individual participants included in the study. Participant demographics are reported in Table 1.

### Study Conditions

Students with ASD were assigned to treatment and control conditions. The first participant we recruited was assigned to the experimental condition so that we could pilot intervention procedures. The other ten participants were randomized to the experimental or control condition using a random

number generator. Randomization occurred after students with ASD and potential facilitators were consented.

### Control Condition

We did not provide any training or direction to adults in the control group. We did not direct them to recruit peers. We told them that we wanted to observe what recess typically looked like.

### Experimental Condition

We provided a 1-h training to each adult facilitator (assigned to the treatment group) that focused on how to identify, initially train, and then support peers on a day-to-day basis during recess. Facilitators identified potential peers by asking the student with ASD about preferred peers, and approaching students who interacted positively with the student in the past. In a 45-min meeting with peers, the facilitator introduced him or herself, described the rationale for the intervention, provided background about the focus student, shared five strategies to engage the focus student, described that he or she would provide support each day at recess, discussed appropriate language and confidentiality, and invited questions from peer. The five strategies were grounded in PRT (Pierce and Schreibman 1997) and had been piloted in a prior study (Harper et al. 2008). They included (a) get your buddy to look at you; (b) ask your buddy to play something with you; (c) show and talk about how to play; (d) compliment your buddy; and (e) if you can't play at the same time, take turns. The facilitator described each strategy, provided examples, modeled it, answered any questions, directed the peers to practice through role play, and delivered specific praise and corrective feedback. After the initial training, facilitators provided ongoing support at recess by talking with peers about their new roles, modeling how they might interact with the target student, and referencing the five strategies (either verbally or using a visual support that we provided). Facilitators were required to maintain the intervention for at least 5 weeks.

### Procedural Fidelity

We measured fidelity of the initial meeting with peers with a 50-item implementation checklist. If the facilitator missed a step, a member of the research team delivered corrective feedback; therefore, fidelity at these initial meetings was 100%. During recess observations, we measured how often peers used one of the five strategies associated with the intervention peers, and how often the facilitator used the facilitation strategies described above. On average, each group of peers used 3.3 strategies per observation during the treatment condition; no peers used any of the strategies

**Table 1** Focus student, peer partner, and adult facilitator demographics

|   | Students with autism    |                           | Trained peers ( <i>n</i> = 19) | Adult facilitators      |                           |
|---|-------------------------|---------------------------|--------------------------------|-------------------------|---------------------------|
|   | Control ( <i>n</i> = 5) | Treatment ( <i>n</i> = 6) |                                | Control ( <i>n</i> = 5) | Treatment ( <i>n</i> = 6) |
| <b>Gender</b>                               |                         |                           |                                |                         |                           |
| Male  | 5 (100.0%)              | 5 (83.3%)                 | 8 (42.1%)                      | 0 (0.0%)                | 0 (0.0%)                  |
| Female                                      | 0 (0.0%)                | 1 (16.7%)                 | 11 (57.9%)                     | 5 (100.0%)              | 6 (100.0%)                |
| <b>Grade level</b>                          |                         |                           |                                |                         |                           |
| Second                                      | 0 (0.0%)                | 1 (16.7%)                 | 3 (15.8%)                      | –                       | –                         |
| Fourth                                      | 1 (20.0%)               | 1 (16.7%)                 | 3 (15.8%)                      | –                       | –                         |
| Fifth                                       | 2 (40.0%)               | 1 (16.7%)                 | 4 (21.1%)                      | –                       | –                         |
| Sixth                                       | 2 (40.0%)               | 3 (50.0%)                 | 9 (47.4%)                      | –                       | –                         |
| <b>Age</b>                                  |                         |                           |                                |                         |                           |
| 8   | 0 (0.0%)                | 1 (16.7%)                 | 3 (15.8%)                      | –                       | –                         |
| 10  | 1 (20.0%)               | 2 (33.3%)                 | 2 (10.5%)                      | –                       | –                         |
| 11  | 2 (40.0%)               | 1 (16.7%)                 | 6 (31.6%)                      | –                       | –                         |
| 12  | 2 (40.0%)               | 2 (33.3%)                 | 8 (42.1%)                      | –                       | –                         |
| <b>Race/ethnicity</b>                       |                         |                           |                                |                         |                           |
| European American                           | 2 (40.0%)               | 4 (66.7%)                 | 17 (89.5%)                     | 5 (100.0%)              | 5 (83.3%)                 |
| African American                            | 2 (40.0%)               | 0 (0.0%)                  | 0 (0.0%)                       | 0 (0.0%)                | 1 (16.7%)                 |
| Hispanic or Latino/a                        | 0 (0.0%)                | 0 (0.0%)                  | 1 (5.3%)                       | 0 (0.0%)                | 0 (0.0%)                  |
| Other or multiple                           | 1 (20.0%)               | 2 (33.3%)                 | 1 (5.3%)                       | 0 (0.0%)                | 0 (0.0%)                  |
| <b>Test scores</b>                          |                         |                           |                                |                         |                           |
| IQ standard score <sup>a</sup>              | 83.5 (12.3)             | 76.6 (22.2)               | –                              | –                       | –                         |
| Adaptive skills standard score <sup>b</sup> | 70.0 (19.8)             | 75.5 (22.3)               | –                              | –                       | –                         |
| <b>Role</b>                                 |                         |                           |                                |                         |                           |
| General education teacher                   | –                       | –                         | –                              | 1 (20.0%)               | 2 (33.3%)                 |
| Special education teacher                   | –                       | –                         | –                              | 2 (40.0%)               | 1 (16.7%)                 |
| Paraprofessionals                           | –                       | –                         | –                              | 2 (40.0%)               | 3 (50.0%)                 |
| <b>Education level</b>                      |                         |                           |                                |                         |                           |
| High school diploma                         | –                       | –                         | –                              | 1 (20.0%)               | 1 (16.7%)                 |
| Associate's degree                          | –                       | –                         | –                              | 0 (0.0%)                | 1 (16.7%)                 |
| Bachelor's degree                           | –                       | –                         | –                              | 2 (40.0%)               | 3 (50.0%)                 |
| Master's degree                             | –                       | –                         | –                              | 2 (40.0%)               | 1 (16.7%)                 |

All numbers represent frequency (percentage) with the exception of test scores, which represent the means and standard deviations of standard scores

<sup>a</sup>Scores were taken from student's school record; instruments included KABC-II (1 in control and 1 in treatment), GARS-3 (1 in control), KBIT-3 (1 in control and 1 in treatment) WISC-IV (2 in control and 3 in treatment), and the UNIT-2 (1 in treatment)

<sup>b</sup>Scores were taken from student's school record; instruments included, GARS-3 (1 in treatment), ABAS-3 (2 in control and 1 in treatment), and Vineland III (2 in treatment). Measures of adaptive skills were not available for 5 students

in the control condition. Each facilitator used at least one facilitation strategy during each observation in the treatment condition; no adult used any facilitation strategies in the control condition.

## Dependent Measures

### Recess Observations

All data were collected through live observation. We observed recess five times for each student (i.e., one time

each week for 5 weeks). Length of recess ranged 20–30 min; we observed from the time the students entered the playground until they lined up to re-enter the school. For students in the experimental group, this included one observation prior to intervention and four observations after intervening. We observed two key variables using a partial interval recording system that involved alternating between 10 s of observation and 10 s of recording. We calculated the percentage of intervals in which a behavior occurred. First, we measured interactions, defined as verbal or nonverbal (e.g., gestures, signs) communicative behaviors directed from the

student with ASD toward his peers, or from peers toward the student with ASD. This included interactions with peers who were and who were not trained by the facilitator. Second, we measured quality of play, for which we developed four mutually exclusive codes. *Appropriate peer play* involved behavior that (a) was actively or passively related to a play activity with peers, (b) was consistent with recess rules and (when applicable) the rules associated with a game, and (c) did not solely revolve around a stereotypic behavior. Examples include playing tag, basketball, or playing on a jungle gym with peers. *Appropriate solitary play* differed from the above definition in that the student was not actively or passively engaged in a play activity with peers. Examples include swinging, solitary pretend play, or shooting a basketball by oneself. *Inappropriate play* involved (a) behavior that was inconsistent with recess rules or (when applicable) the rules associated with a game, or (b) stereotypic behavior in isolation of any behavior that would fall into the aforementioned play categories (e.g., hand flapping, body rocking, or repeating the same phrase over and over). *No play* was defined as not engaging in any play category that could be categorized into any of the aforementioned categories. Examples include sitting on a bench or standing in one place without moving or interacting.

### Observer Training

Observers included two doctoral students and one undergraduate student in special education. Before collecting data, observers were required to score 100% on a written test of

coding definitions, 90% agreement on all variables with the first author when coding a training video, and 90% agreement with the first author at a live observation.

### Interobserver Reliability

Interrater reliability was computed on 33.3% of observation session. Across all variables, average point-by-point agreement was 99.0%. Agreement never fell below 87.0% for any dependent variable during any individual observation session.

### Social Validity Questionnaires

We gauged perceptions of the intervention by surveying adult facilitators and peers, and interviewing students with ASD. Facilitator questions are reported in Table 2, and peer and student with ASD questions are reported in the Results section. Questions were adapted from a social validity instrument developed by Asmus et al. (2017).

### Data Analysis

We used multi-level modeling to test for group differences, with one model for each of the seven dependent variables (see Table 3). Five data points (i.e., one for each observation) were nested within each student. Repeated observations over time (i.e., *time*) were the level-1 variable, and were assigned a dummy variable reflecting pre- or post-intervention. *Time* was nested within student

**Table 2** Results from facilitator social validity survey

| Social validity items  | Scores    |
|--|-----------|
| As a result of this strategy, the student with autism interacted more with peers during recess | 4.2 (0.4) |
| As a result of this strategy, the student with autism played more appropriately at recess      | 3.8 (1.1) |
| As a result of this strategy, the student with autism made more friends                        | 4.2 (1.3) |
| Peers enjoyed providing support to the student with autism                                     | 4.8 (0.4) |
| Overall, I enjoyed participating in this project   | 5.0 (0.0) |
| The amount of time required to use this strategy was reasonable                                | 4.6 (0.5) |
| I feel I was effective in my role as a facilitator   | 4.4 (0.5) |
| I would need ongoing consultation to keep implementing this strategy                           | 2.2 (0.4) |
| Implementation of this strategy required considerable support from other school staff          | 2.2 (1.1) |
| I am motivated to continue using this strategy   | 4.6 (0.5) |
| I would not be interested in implementing this strategy again                                  | 1.2 (0.4) |
| This strategy was a good way to address the social needs of the student with autism            | 4.4 (0.5) |
| This strategy is a good fit for recess at this school  | 4.6 (0.9) |
| I understand the procedures of this strategy   | 4.8 (0.4) |
| I would know what to do if I were asked to implement this strategy again                       | 4.6 (0.5) |
| I would use this strategy with other students  | 4.8 (0.4) |

The six facilitators in the experimental group rated each item on a 5-point Likert-type scale (i.e., 1 strongly disagree, 2 disagree, 3 neither agree nor disagree, 4 agree, 5 strongly agree)

Scores are reported in terms of the group mean and standard deviations

**Table 3** Effects of the peer-mediated PRT

| Dependent variable          | Indep. variable | Estimate | SE   | <i>t</i> ratio | <i>df</i> | <i>p</i> value | Pooled SD | ES <sup>a</sup> |
|-----------------------------|-----------------|----------|------|----------------|-----------|----------------|-----------|-----------------|
| Total interactions          | Intercept       | 0.28     | 0.09 | 3.04           | 40        | 0.004          |           |                 |
|                             | Time            | 0.00     | 0.12 | -0.01          | 40        | 0.996          |           |                 |
|                             | Condition       | -0.14    | 0.09 | -1.68          | 42        | 0.101          |           |                 |
|                             | Time*condition  | 0.32     | 0.12 | 2.78           | 42        | 0.008          | 0.28      | 1.13            |
| Focus student contributions | Intercept       | 0.20     | 0.09 | 2.24           | 32        | 0.033          |           |                 |
|                             | Time            | 0.02     | 0.12 | 0.18           | 32        | 0.855          |           |                 |
|                             | Condition       | -0.10    | 0.08 | -1.30          | 42        | 0.200          |           |                 |
|                             | Time*condition  | 0.23     | 0.10 | 2.23           | 42        | 0.031          | 0.23      | 1.01            |
| Peer contributions          | Intercept       | 0.18     | 0.07 | 2.60           | 37        | 0.013          |           |                 |
|                             | Time            | -0.09    | 0.06 | -1.35          | 42        | 0.186          |           |                 |
|                             | Condition       | 0.04     | 0.10 | 0.46           | 37        | 0.646          |           |                 |
|                             | Time*condition  | 0.18     | 0.09 | 2.08           | 42        | 0.043          | 0.20      | 0.89            |
| Appropriate peer play       | Intercept       | 0.22     | 0.15 | 1.47           | 32        | 0.153          |           |                 |
|                             | Time            | -0.03    | 0.13 | -0.19          | 38        | 0.847          |           |                 |
|                             | Condition       | 0.13     | 0.21 | 0.60           | 32        | 0.551          |           |                 |
|                             | Time*condition  | 0.26     | 0.19 | 1.41           | 38        | 0.167          | 0.29      | 0.89            |
| Appropriate solitary play   | Intercept       | 0.61     | 0.16 | 3.71           | 32        | 0.001          |           |                 |
|                             | Time            | -0.04    | 0.15 | -0.26          | 38        | 0.798          |           |                 |
|                             | Condition       | -0.07    | 0.23 | -0.32          | 32        | 0.750          |           |                 |
|                             | Time*condition  | -0.10    | 0.21 | -0.49          | 38        | 0.626          | 0.35      | -0.29           |
| Inappropriate play          | Intercept       | 0.06     | 0.09 | 0.65           | 36        | 0.518          |           |                 |
|                             | Time            | 0.06     | 0.08 | 0.76           | 38        | 0.455          |           |                 |
|                             | Condition       | 0.05     | 0.13 | 0.36           | 36        | 0.718          |           |                 |
|                             | Time*condition  | -0.16    | 0.12 | -1.39          | 38        | 0.174          | 0.13      | -1.22           |
| No play                     | Intercept       | 0.05     | 0.06 | 0.83           | 48        | 0.409          |           |                 |
|                             | Time            | 0.07     | 0.07 | 0.98           | 48        | 0.333          |           |                 |
|                             | Condition       | -0.03    | 0.09 | -0.32          | 48        | 0.749          |           |                 |
|                             | Time*condition  | -0.06    | 0.10 | -0.65          | 48        | 0.521          | 0.08      | -0.72           |

SE standard error, *df* degrees of freedom

<sup>a</sup>Cohen's *d* was calculated by dividing unstandardized regression coefficients by the pooled standard deviation of the dependent variable. Degrees of freedom were obtained by a Satterthwaite approximation

(level 2). We also included *condition* (i.e., experimental condition) and a *time\*condition* interaction in the model. In this type of model, the interaction term (i.e., *time\*condition*) is the intervention effect of interest; the *condition* term captures group differences at baseline, and the *time* term capture change over time that cannot be attributed to the treatment. We analyzed the data in SPSS using the MIXED command. Random terms could not be estimated due to the small sample size; therefore, only fixed effects were estimated. We also calculated Cohen's *d* for each interaction effect by dividing the correlation coefficient by the pooled standard deviation. Given that this was a pilot feasibility study, we did not adjust the alpha for multiple comparisons.

## Results

Results of the multi-level modeling analysis are reported in Table 3. We detected statistically significant (i.e.,  $p < .05$ ) and substantial intervention effects on total interactions ( $d = 1.13$ ), interactions from the target student toward peers ( $d = 1.01$ ), and interactions from peers toward the target student ( $d = 0.89$ ). Although we did not detect statistically significant intervention effects on quality of play, effect sizes were substantial. These included an increase in *appropriate peer play* ( $d = 0.89$ ), a decrease in *appropriate solitary play* ( $d = -0.29$ ), a decrease in *inappropriate play* ( $d = -1.22$ ), and a decrease in *no play* ( $d = -0.72$ ).

The six facilitators in the experimental group were asked a series of questions to which they responded on a 5-point Likert scale that ranged from ‘strongly disagree’ to ‘strongly agree’; for a complete list of items, scaling, and results, see Table 2. Facilitators most strongly agreed with the statements that *peers enjoyed providing support to the student with autism; overall, I enjoyed participating in this project; I understand the procedures of this strategy; and I would use this strategy with other students*. Facilitators most strongly disagreed with the statements that *I would need ongoing consultation to keep implementing this strategy, implementation of this strategy required considerable support from other school staff, and I would not be interested in implementing this strategy again*.

The six students with ASD in the experimental group were asked a series of questions to which they responded ‘yes’, ‘no’, or ‘not sure’. All six students with ASD said that they liked going to recess, 5 said they enjoyed playing with the trained peers (1 ‘no’), 3 said that the trained peers taught them new ways to play (2 ‘not sure’; 1 ‘no’), 5 students said they considered the trained peers to be their friends (1 ‘no’), and all 6 students said they would like to keep hanging out with the trained peers. When asked if they had anything else to share, one student said, “Sometimes I was alone and didn’t know who to play with and they would come up and ask me to play.” Another student said, “I played tag with [names of trained peers]. They taught me to never give up and they helped me to get to the bases.”

Peers were asked a series of questions to which they responded ‘agree’, ‘disagree’, or ‘not sure’. All 19 peers agreed that they enjoyed supporting their buddy at recess, all 19 agreed they would be willing to be a peer buddy again in the future, and 14 agreed they would recommend being a peer buddy to other students in the future (5 said ‘not sure’). When asked what they liked best about supporting their peer buddy, one student said, “I liked to see how he played games with me and to hear what he named his games. I also liked to play his games.” Another student said, “I liked seeing him actually make new friends.” Another student said, “I liked playing soccer with him. He was really good.” When asked if there was anything that they did not like about being a peer buddy, 16 peers said “no”. The other three peers said, “sometimes I had to miss out on other things”, “I felt kind of like I had to be with him the whole time”, and “sometimes I wanted to play with my other friends when he wanted to play.”

## Discussion

We piloted a practitioner-implemented peer-mediated intervention focused on improving social outcomes for students with ASD at recess. We found that the intervention

significantly and substantially increased interactions between students with ASD and their peers. Improvements in the quality of play were large, but not statistically significant. Stakeholders provided positive feedback about the feasibility of the intervention and their perception of its effects. These findings extend the literature in a number of key ways.

First, school staff can feasibly facilitate peer-mediated PRT at recess. This extends findings from a previous study that involved direct facilitation by an external research team. We found it particularly encouraging that staff who were already supervising recess reported enjoying implementing the intervention, perceived it to be effective, and would be willing to facilitate peer-mediated PRT with other students in the future.

Second, staff-implemented peer-mediated PRT are an effective way to increase interactions between students with ASD and their peers. Effects for peer interactions were statistically significant and substantial, and both students with ASD and their peers reported that they enjoyed interacting with one another. Based on these results, combined with previous findings from a single-case design study (i.e., Harper et al. 2008), peer-mediated PRT is a promising means to increase social interactions between students with ASD and their peers at recess.

Third, staff-implemented peer-mediated PRT might be an effective way to improve quality of play for students with ASD, but more research is needed. Although there was a substantial effect size for increased *appropriate peer play*, our limited statistical power did not enable us to detect a statistically significant effect. However, the large increase in appropriate peer play is promising, and this outcome has not been previously measured in published studies.

## Limitations and Directions for Future Research

Findings from this study are limited by a small sample that might not be representative of the population. For example, most students were male and White. A related limitation is that variance estimates are less reliable in multi-level models with relatively few level-two clusters (McNeish and Stapleton 2016). Therefore, we recommend that the reader interpret the statistical tests with caution. These limitations could be addressed in a large, fully-powered randomized-controlled trial. In addition, we only found statistically significant effects for peer interactions, which was the most proximal dependent variable. If researchers in future studies had additional statistical power, they might be able to detect significant improvements in more distal outcomes.



## Conclusion

Findings from this study provide promising evidence that school staff can feasibly and effectively facilitate peer-mediated PRT to improve social outcomes for students with ASD at recess. Peer-implemented PRT is an exciting avenue to work toward building social connections and friendships between students with ASD and their peers.

**Author Contributions** MEB conceived of the study, designed the study, conducted the analysis, and drafted the manuscript; SAD coordinated implementation of study procedures, collected data, and helped to draft the manuscript; MAB collected data and helped to draft the manuscript. All authors read and approved the final manuscript.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Research Involving Human Participants** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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