The Effectiveness of Animal Assisted Therapy in Adults with Autism Spectrum Disorder: Study Protocol for a Randomized Controlled Trial

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Abstract

Introduction: Comorbid problems are common in adults with autism spectrum disorder (ASD). The number of studies on the effects of behavioural interventions in adults with ASD is limited, the needs of adults with ASD differ from individual to individual, and the numbers of ASD diagnoses are on the rise worldwide. For this reason, it is of great importance to develop new interventions for adults with ASD and to examine the effectiveness of those interventions on reducing comorbid symptoms.

In children with ASD, preliminary positive results are found in interaction with an animal. The effects of Animal Assisted Therapy (AAT) have not yet been studied in adults with ASD. Therefore, this study is focused on the effects of AAT in adults with ASD, without intellectual disability.

Methods: The explorative study will be conducted in adults with ASD and aims to provide insight into the effects and feasibility of AAT. The intervention consists of ten weekly one-on-one sessions conducted in the presence of a dog. Participants in this study will be randomized after the baseline measurement and equally distributed towards the AAT or waiting-list control condition. The study outcome measures are self-reported stress, psychological distress, self-confidence, levels of autistic traits, heart rate variability, salivary cortisol, and α-amylase. Data will be analysed with mixed models. A comprehensive process evaluation will be conducted for more insight into barriers and facilitators and into feasibility of the intervention. The medical ethics committee CMO region Arnhem-Nijmegen, the Netherlands, approves this study. Written informed consent will be obtained from all participants before enrolment.

Discussion: This study protocol describes a research designed to explore the effects and feasibility of Animal Assisted Therapy in adults with autism spectrum disorder.

Keywords: Autism spectrum disorder; Animal assisted therapy; Service dog; Adults

Abbreviations AAA: Animal Assisted Activities; AAE: Animal Assisted Education; AAI: Animal Assisted Interventions; AAT: Animal Assisted Therapy; ADI-R: Autistic Disorder Interview-Revised; ASD: autism spectrum disorder; CBT: Cognitive Behavioural Therapy; DSM-5: Diagnostic and Statistical Manual of Mental Disorders 5th edition; IAHIAIO: International Association of Human-Animal Interaction Organizations; MBRS: Mindfulness-Based Stress Reduction; PSS: Perceived Stress Scale; RCT: Randomized Controlled Trial; RSES: Rosenberg Self-Esteem Scale; SCL-90-R: Symptom Checklist-90-Revised; SRS-A: Social Responsiveness Scale for Adults; T0-T2: Data Collection Time Points 0-2; T0, T30, T60: Data Collection Time Points (in min)

Introduction

Autism spectrum disorder (ASD) is characterized by two main criteria: (A) persistent deficits in social communication and social interaction across multiple contexts and (B) restricted, repetitive patterns of behaviour, interests, or activities according to the DSM-5 criteria [1]. The prevalence of childhood ASD is estimated at 1% [2,3]. A similar prevalence is found in the adult population in England [4]. Over the past 30 years, the prevalence of ASD has increased substantially, which has been attributed in part to the increased awareness and recognition of ASD and the change of diagnostic concepts and criteria [5,6].

In addition to the problems experienced by people with ASD as described in the diagnostic criteria of the DSM-5, many comorbid problems have also been reported, the most common of which are anxiety and mood disorders. Research has shown that around 50% of adults with ASD meet the criteria of an anxiety or mood disorder [7-9]. In adults with ASD, a positive correlation is found between levels of anxiety and stress and in particular, with a decreased ability to cope with change, anticipation, sensory stimuli and unpleasant events [10]. Also common in people with ASD are sleeping problems, low self-esteem, a negative self-image, and rumination [11,12].
Only limited research has been done on the effectiveness of interventions in adults with ASD, which aim to reduce comorbid problems such as anxiety and mood disorders. To the best of our knowledge, no effects of reduction in depression and anxiety are described as a result of psychotropic medication in a large sample size of adults with ASD, without intellectual disability. Considering the non-psychopharmacological treatments, positive effects are found in cognitive behavioural therapy (CBT) and mindfulness based stress reduction (MBSR) [13-17]. And while there is some evidence for long-term results, empirical evidence is limited by the small number of subjects involved in these studies [18,19]. Although there are positive effects reported as a result of CBT and MBSR interventions, they might be not being suitable for everyone. For example, the concept of CBT can be difficult to grasp and generalizing the learned techniques into daily life situations may pose a real challenge; MBSR requires frequent practice, and implementation of the exercises into the routines of daily life can be difficult [20,21].

The adult population is a highly understudied population compared to children with ASD, especially translational treatment research [22]. With the markedly increased reports of ASD diagnoses during the past three decades, the high levels of distress associated with ASD, and the heterogeneity of the population, creating effective interventions that reduce comorbid problems is of great importance.

Animal Assisted Interaction (AAI) can be promising as an effective intervention in adults with ASD. In a recent review study, Beetz et al. show that interaction between humans and animals improves mental and physical health [23]. For example, improvements in human social behaviour are one of the core problems in ASD. Furthermore, they show a reduction in stress-related parameters as well as a reduction of self-reported anxiety levels [24-27], other main problems in ASD. AAI is used as an umbrella term that includes Animal Assisted Therapy (AAT), Animal Assisted Activities (AAA) and Animal Assisted Education (AAE). The International Association of Human-Animal Interaction Organizations (IAHAIO) has written a white paper that includes the following international definitions of AAI and AAT [28].

AAI is a goal-oriented intervention that intentionally includes or incorporates animals in health, education and human service for the purpose of therapeutic gains in humans. Animal Assisted Therapy is a goal-oriented, planned and structured therapeutic intervention directed and/or delivered by health, education and human service professionals. Intervention progress is measured and included in professional documentation. AAT is delivered and/or directed by a formally trained (with active licensure, degree or equivalent) professional with expertise within the scope of the professionals’ practice. AAT focuses on enhancing physical, cognitive, behavioural and/or socio-emotional functioning of the particular human client.

The literature does include some studies on the effects of AAI in children with ASD. A review by O’Haire describes increased social interaction and communication skills, while stress parameters, autistic severity, and problem behaviour decreased as a result of interactions with an animal [29]. Focusing on studies in children with ASD and interactions with dogs, Martin and Farnum mention that children are more playful, more focused, and more aware of their social environment when there is a living dog present, as compared to a stuffed toy or an object [30] and Prothmann et al. found that children have longer interactions with a dog than with a person or object [31]. Sams et al. note more social interaction and language use among children with ASD in therapy involving a dog [32]. Berry et al. review overlapping studies on AAI involving dogs and children with ASD and conclude that interactions with a dog can help children with ASD overcome inabilities to interact with other people [33]. In studies with therapy programs of horseback riding for children with ASD, Bass et al. found improvements in sensory integration and sensitivity, as well as improvements in social motivation and attention in a twelve-week therapy program of horseback riding compared to a waiting-list control group [34] and in a six-month program using horses during therapy for children with ASD, Borgi et al. observed improvements in the social and executive functioning of the participants, compared to children with ASD in a waiting-list control group [35].

Physiological effects of animals on children with ASD have also been explored. Focussing on cortisol, Viau et al. report a reduced cortisol awakening response in children with ASD when a service dog is placed at home [36] and Tabares et al. describe a reduction in saliva cortisol in children with ASD after four sessions of therapy with horses [37].

Mechanisms explaining the effects of animals on pro-social behaviour vary from a social catalyst effect of animals on humans, to a social support effect of dogs on humans. Animals can provide a comforting and supporting environment, where humans can experience a consoling place to talk and touch. Another theory is that animals have a modeling effect on humans. They provide immediate feedback, which may help humans learn appropriate social behaviour including the relationship between cause and effect. Through role assumption, people are able to fulfil the role of teacher or caretaker in a human-animal interaction and may therefore display more nurturing behaviour [28,38-40]. On a physiological level, increases in pro-social behaviour are explained by, for example, an increase in oxytocin resulting from human-animal interaction [23,26,37].

The results in these studies may be promising but should be interpreted with caution because of limitations in the methodology. Most of the studies have small sample sizes, different outcome variables, no control group or lack of randomization, and lack a standard methodology. Therefore, results are difficult to compare. Additionally, the literature provides poor descriptions of the intervention, the origin and training of the assistance animal, and the education of the therapist. Additional research that satisfactorily addresses these criteria is recommended.

Despite the positive (preliminary) results on AAI in children with ASD, the effects of such an intervention have not yet been studied in adults with ASD. Since there are many similarities in the problems experienced by both children and adults with ASD, it may be expected that AAT can also be effective in improving mental health among adults with ASD. Before conducting a hypothesis testing study, it is important to examine the feasibility and implementability of such an intervention have not yet been studied. Before conducting a hypothesis testing study, it is important to examine the feasibility and implementability of such an approach in a pilot study [41]. The aim of this study is to extensively document an AAT intervention in adults with ASD. In combination with a comprehensive process evaluation of the intervention, we will explore potential effects (and their confidence intervals) of AAT in adults with ASD on comorbid problems, such as psychological distress, stress, self-confidence, and autistic traits.

Study Objectives

Self-reported stress will be measured with the Perceived Stress Scale (PSS) [42,43]. The PSS contains ten items, rated on a five-point Likert scale, ranging from 0 ‘never’ to 4 ‘very often’. This instrument has a good internal consistency and an adequate convergent validity [43-46].
Overall distress will be measured with the Symptom Checklist-90-Revised (SCL-90-R) [44]. The SCL-90-R contains 90 items, each rated on a five-point Likert scale ranging from 1 ‘none’ to 5 ‘very severe’. This questionnaire has 9 subscales, and includes subscales such as ‘anxiety’ and ‘depression’. The original SCL-90-R and the Dutch version of this instrument have an excellent reliability and construct validity [47,48].

Self-esteem will be measured with the Rosenberg Self-Esteem Scale (RSES) [49]. The RSES contains 10 items, each rated on a four-point Likert scale ranging from ‘very untrue’ to ‘very true’. This instrument has a high validity and test-retest reliability [50,51].

Autistic traits will be measured by the Dutch version of the Social Responsiveness Scale for Adults (SRS-A) [52]. This instrument has two versions, one for the participant and another that can be completed by an informant. The SRS-A contains 64 items, each rated on a four-point Likert scale ranging from ‘very untrue’ to ‘very true’. The adult version of this instrument has not yet been evaluated. The child version of this instrument is rated with a high validity and reliability [53].

Physiological stress will be measured by saliva cortisol, saliva α-amylase and heart rate variability. Heart rate variability is measured with the VU-AMS 4.6 device [54,55].

Methods

Design

The present study is designed as an explorative non-blind randomized controlled trial (RCT) with two conditions (intervention versus waiting-list control). After recruiting and screening for eligibility, a baseline assessment (T0) will be conducted, and participants will be randomized, blindly for the principal researcher, to either the intervention condition or the waiting-list control condition using a computer program. Furthermore, a post-intervention assessment after ten weekly therapy sessions (T1) and a ten-week follow-up assessment (T2) will be conducted. Participants in the intervention condition will also have a physiological assessment at five weeks after the baseline assessment (during the fifth intervention session). Six participants in the intervention condition will be videotaped during sessions one, five and ten. The study design is presented in Figure 1. Participants in the waiting-list control condition will have the possibility to receive the intervention after the last assessment T2.

Recruitment

Outpatients from the mental health care organization GGZ Oost Brabant diagnosed with ASD are recruited by oral information from their therapists and information folders in the waiting room. Potential participants receive written and verbal information about the study. After giving their passive consent, participants are screened by the research team for inclusion and exclusion criteria. Participants who meet the inclusion criteria receive a written informed consent and are invited to an intake to establish treatment goals.

Sample size

This mixed methods pilot study will be conducted in 72 participants (36 in experimental and 36 in control condition). In a two groups two time-points study, between 30 and 40 participants per group is considered sufficient for providing preliminary information on a between-group effect-size and yielding confidence intervals for a subsequent power analysis in future hypothesis testing studies [45].

With 72 participants, a dropout of 20% can be still allowed to reach 30 participants per group, although the dropout of 20% is an overestimation for the target population with ASD. Studies on behavioural interventions in adults with ASD showed dropouts varying between 0 and 14% [18,19,21].

Animal assisted therapy

The AAT program consists of ten weekly one-on-one sessions, with duration of one hour per session. In this study, the therapy will be given by three different therapists. To reduce individual differences in therapy, a protocol will be used. The structure of the sessions and the therapy protocol is described in the next two paragraphs. Detailed information is available in Tables 1 and 2.

The therapy dog present in the therapy sessions is a trained service dog from the Dutch foundation ‘Stichting Hulphond’. The physical and mental health of the dogs participating in this study is strictly monitored by the service dog foundation. The therapy dogs are under supervision of a dog trainer and have regular veterinary check-ups. The therapists participating in this study all have a college or university degree in mental health care and have specialized knowledge and experience in working with ASD. Additionally, they had completed courses, selected by the service dog foundation, in dog behaviour and mental and physical behaviour in dogs.

During the therapy the dog will not be on a leash, with an exception for the exercises in the open air and the preparation for walking in the
open air. There is a mat on the floor were the dog can rest and water available in the therapy room where the dog has constantly access to.

Therapy program

During the first three sessions of the AAT program, participants will learn how to approach the dog and how they can make contact with the dog. Participants will learn how to instruct the dog (verbally and non-verbally) through basic commands and leadership exercises, how to behave appropriately with the animal, and how they can interact (verbally and non-verbally) with the dog. Furthermore, they will learn how to reward the dog for good behaviour, and what to do when it behaves in an undesirable way. In sessions four through six, the main focus of the therapy sessions is on recognition and interpretation of behaviour signals (verbal and non-verbal) of the dog, and on signaling and interpretation of their own behaviour, feelings and thoughts (stress and basic emotions). The focus during these sessions is mainly on the interaction between the dog and the participant's behaviour, feelings and thoughts. For example, they learn about cause-and-effect: how they can influence behaviour of another living being through their own behaviour (verbal and non-verbal) and vice versa. In sessions seven through nine, the exercises will be expanded by the following themes: sensory stimuli, attention, and concentration. In the tenth session, therapy goals will be evaluated and participants are coached in how to maintain the learned skills and incorporate them into their daily lives.

Structure of the sessions

The first five min of the therapy session the therapist and participant will discuss how the participant is feeling and if and how the participant applies the learned skills from the previous session into his/her daily life.

Next, exercises from the previous session will be repeated. The therapist will observe the potential differences in execution of the exercises and reflects on the learned skills together with the participant. Thereafter, new exercises will be introduced. The therapist again will reflect on the execution of the exercise together with the participant, using the observed behaviours of the participant and the dog and the interaction between them. The participant's thoughts and feelings will also be discussed. During the final 10-15 min there will be time for physical interaction with the dog: petting, cuddling or grooming. The participant will be asked to sit down at the floor or in a chair, and has the choice to interact with the dog, or to sit down without physical interaction. When the dog is walking away from the interaction while the participant does want to interact, this will be discussed with the therapist. The therapist will ask how the participant is feeling or what he or she is thinking and will give feedback on the interaction or give advice to facilitate the interaction.

Before leaving the therapy room, the therapist and participant will discuss again how the participant is feeling and how he or she can implement the learned skills in his or her daily life.

| Time       | Structure                                                                 |
|------------|--------------------------------------------------------------------------|
| 0-5 min    | Welcome                                                                  |
| 5-15 min   | Discuss how the participant is feeling and if and how he/she applied the learned skills into his/her daily life |
| 15-45 min  | Repeating exercises from the previous session                           |
| 45-55 min  | New exercises                                                            |
| 55-60 min  | Physical interaction with the dog; petting, cuddling or grooming the dog or resting in the therapy room without physical interaction |
| 55-60 min  | Discuss how the participant is feeling and how he/she can apply the learned skills into his/her daily life |
| 60-65 min  | Saying goodbye                                                           |

Table 1: Structure of the sessions.

| Session | Protocol                                                                 |
|---------|--------------------------------------------------------------------------|
| 1       | Welcome                                                                  |
|         | Introduction to the therapist and dog                                    |
|         | Basic rules about interaction with the dog                               |
|         | Explanation and practice with rewarding and ignoring behaviour          |
|         | Basic commands (verbal and non-verbal) (sit, down, shake hands, high five)|
|         | Basic following/leadership exercise                                    |
| 2       | Repeating basic commands and following/leadership exercise               |
|         | Advanced following/leadership exercise                                  |
|         | Learning by trial and error exercise                                    |
### Table 2: AAT protocol.

| Page | Procedure |
|------|-----------|
| 3    | Repeating learning by trial and error exercise  
Advanced trial and error exercises (leadership, timing, attention, emotions) |
| 4    | Repeating advanced trial and error exercises  
Introduction to non-verbal communication and body language of dogs and humans  
Practice with non-verbal communication and body language between humans and dogs  
Introduction to emotions of humans  
Practice with emotions and effects of emotions and body language on communication/behaviour and stress  
Practice with executive functioning in playing and learning new skills to the dog |
| 5    | Repeating emotion exercise  
Repeating executive functioning exercise  
Introduction to levels of stress  
Practice with signalling stress and ‘saying no’  
Practice with boundaries (verbal and non-verbal) |
| 6    | Repeating boundary exercises  
Introduction to social skills and differences between humans  
Practice with social skills and cause-and-effects (effects of own body language, stress levels and verbal language on dogs and vice versa) |
| 7    | Repeating social skills exercises  
Advanced executive functioning exercise  
Attention exercise  
Preparation of walking outside (practice with walking with a leash) |
| 8    | Repeating walking with a leash  
Walking outside (attention, concentration, sensory stimuli, stress)  
Attention, concentration and memory exercise |
| 9    | Repeating attention, concentration and memory exercise  
Repeating walking outside with more sensory stimuli |
|      | Repeating walking outside  
Discussion of learning goals  
Coaching how to maintain the learned skills and incorporate them into the daily live  
Repeating the most favourite exercise  
Saying goodbye |

### Procedure

Saliva samples and heart rate variability are collected during T0, T1 and T2 for both the intervention and waiting-list control condition. Two saliva samples are taken with a Sarstedt Salivette (article number 51.1534.500) in a 30 min time frame. First, the physiological measures of stress will be collected and then participants will be asked to fill in the questionnaires. When participants arrive at the setting where the measurement will be completed, participants will be connected to the VU-AMS device (heart rate variability measurement) at first, then participants will be asked to sit down and saliva will be collected (t0). The physiological measurements will be done in a poor stimulus laboratory setting, while participants are reading magazines in silence. A second saliva sample is collected 30 min after the first saliva sample is taken (t30). Then, the VU-AMS will be disconnected, and participants are asked to fill in the questionnaires. To minimize the effect of circadian rhythm of cortisol, participants will be measured at the same day and hour at T0, T1 and T2. Two saliva samples are taken:
at the beginning and the end of the physiological measurement, because saliva cortisol has a delay in response time compared to serum cortisol after exercise. Some participants may be stressed or have done some exercise (for example cycling) to get to the measurement location. To minimize the effects of such factors, two samples will be collected and the sample with the lowest value will be used for analysis. Collected saliva will be immediately stored in a cool box for a maximum of four hours. The salivates will be labeled with a code so the analyst at the laboratory can analyze the samples blindly. The samples are stored at -20°C after the assessment and brought to the laboratory where they are stored for a maximum of 90 days at -80°C. The samples are centrifuged and analyzed by a laboratory assistant.

After the physiological measurements are completed, participants are asked to fill in the questionnaires at T0, T1 and T2. When the questionnaires are completed, participants in the intervention group are also asked to answer several questions on paper for process evaluation during T1.

To investigate the effect of a therapy session on physiological stress in the intervention group, physiological measurements will be conducted during session five in a 60 min time frame. To compare levels of stress at the beginning and the end of a therapy session, saliva is collected at the beginning of the session (t0) and after 60 min at the end of the session (t60). The last 15 min of the therapy session participants are asked to pet the dog. The second measurement (t60) will therefore, provide some additional information on the effects of physical interaction with a dog. This measurement will be compared to the baseline measurement t0. Heart rate variability is measured throughout the fifth session (60 min) to assess stress during a therapy session.

Six participants will be videotaped during sessions one, five and ten.

Results

Structured video analysis

Considering the explorative character of this study, and for generating hypotheses on potential therapeutic mechanisms, we will collect observational data on behavioural changes (e.g. in verbal initiative, confidence in posture, intonation, smiles, and spontaneous touching and physical contact with the dog). For this reason, video recordings of the therapy sessions will be analysed using a standardized codebook and the computer program Mediacoder [56]. For each prescribed code, two raters will be trained using the same training video fragment independently. Their codes will be compared and the proportion of inter-rater agreement is calculated. An agreement of at least 80% has to be reached before raters will be allowed to code the research data.

Process evaluation

For process evaluation, we use a model that evaluates process data on sampling quality, intervention quality, and implementation strategies [57]. The sampling quality describes the results of the recruitment, randomization, and allocation procedures, and barriers and facilitators to the recruiting of the participants. To gain insight into the quality of the intervention, participants in the intervention condition will be asked to complete a questionnaire at T1 (ten weeks after the baseline), and the therapists will be asked to answer the same questions after accomplishment of the data collection stadium. The questionnaire, developed for this study, contains five questions on a five-point Likert scale about satisfaction of the participants about the intervention, and relevance and feasibility of the intervention. For example ‘Do you think this therapy is relevant for people with ASD?’ (1 is ‘not relevant at all’ and 5 are ‘very relevant’). The questionnaire also contains three open-ended questions about adherence of the participants during the intervention, and barriers and facilitators of the intervention and its implementation in a mental health care organization with outpatients. For example ‘This therapy contains ten sessions. How many therapy sessions have you completed?’

Data Analysis

To analyze the data for the intervention effects, mixed models will be built with two levels: repeated scores of the participant on self-report and physiological measurements (level 1) nested in the participant (level 2). PSS, SCL-90, RSES and SRS-A scores, and levels of cortisol, α-amylase and heart rate variability will be used as outcomes in primary analysis. Initially, missing scores for dropouts will not be imputed. For intention-to-treat analysis, random intercept models will be built for the outcome variables as a dependent variable. The main model adjusted for the baseline measurement includes the intervention condition (intervention yes/no), time points (T1 and T2) and the interaction of time × intervention condition. Models with the interaction will be compared, using likelihood ratio tests, to models without the interaction.

Additional sensitivity analysis will account for age (years), sex (male/female), intelligence (total IQ score), having a dog at home at T0 (yes/no), dropouts (yes/no) and adherence (number of sessions completed during the study), and for interactions of these variables with the intervention condition.

First, we will build the most complete model and then compare models with and without interaction terms. Finally, to find the most reduced model, we will compare models with and without individual factors.

Process evaluation data will be analyzed using descriptive statistics for quantitative data and an appropriate comprehensive guideline for the qualitative research for open-ended questions, such as the Qualitative Analysis Guide of Leuven [58-60].

Discussion and Conclusion

In this study protocol, the design of the explorative study on the effect of Animal Assisted Therapy (AAT) in adults with ASD is presented. The purpose of this study is to gain insight into the effects of AAT in adults with ASD on a broad spectrum of outcomes. The effects on self-reported stress, overall distress, self-esteem and autistic traits will be investigated and reported. Furthermore, the effects on physiological measurements are explored. Additional video analysis and process evaluation will be performed to collect quantitative and qualitative data for more insight into feasibility of the intervention and barriers and facilitators.

Strengths and Limitations

The aim is to gain knowledge about the effects of AAT in adults with ASD. The effects of this intervention have never been investigated in this population. Previous studies on the effect of AAT in children have been limited by study designs with weaknesses, such as lack of a control group, no randomization, a small number of participants, or a research procedure without a strict research protocol. This study...
includes a waiting-list control group, randomization towards one of the conditions after completion of the first assessment, and the description and use of a strict research and therapy protocol.

Data collection will be done on a broad spectrum of outcomes. In addition to exploring commonly used self-reports physiological measurements are also investigated. Qualitative data will also be collected by video analysis and process evaluation.

This study is explorative and is to the best of our knowledge the first study on the effects of AAT in adults with ASD. The intended sample size is a common size for the goal of this study, which is to gain insight into the effects of this intervention in this particular target group.

The effects of this study might also have a bias towards people who like dogs. Therefore, generalizations about the total population of high functioning people with ASD must be made with caution since individuals with anxiety or aversion towards dogs have been excluded from this study.

Declarations

Ethics approval and consent to participate

The medical ethics committee CMO region Arnhem-Nijmegen, the Netherlands, approves this study. NL-number: NL48974.091.14. Written informed consent for participation in the study will be obtained from all participants prior to their baseline measurement at T0.

Trial Registration

Dutch Trial Register NTR 5938; results.

Competing Interests

The authors declare that they have no competing interests.

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Author’s Contributions

CW designed the study and co-designed the intervention, wrote the paper, and is also responsible for data collection, data analysis, and for reporting the study results. AS co-designed the study and co-wrote the paper. RL assisted in the design and statistical analysis plan of the study and co-wrote the paper. ME assisted in the design of the study and co-wrote the paper. TV assisted in the design of the study and co-wrote the paper. All authors have read and approved the final manuscript.

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