The effects of targeted phantom motor execution, prosthetic embodiment, and surgical closure on phantom limb control, and physical function in people with unilateral (single) transtibial (below-the-knee) amputation.

Study Protocol and Statistical Analysis Plan

NCT number: Bio_2855

Document date: July 29th, 2021

Experimental setup

This research is a 2-arm, parallel, open label, non-blinded randomized controlled trial comprised of four independent but interrelated studies with the same participants. The familiarization phase, enrollment verification, obtaining verbal consent to participate, and gathering screening information will be done via phone interview. After screening participants, the Medical and Demographic Questionnaire will be administered. The participant will be e-mailed a link to complete the following surveys/questionnaires: Medical and Demographic questionnaire, Waterloo Footedness Questionnaire to determine their dominant lower extremity, and The Survey of Phantom Limb Awareness and Control that is a commonly used questionnaire regarding phantom phenomena. The participant can use a smartphone, iPad or computer to answer these surveys/questionnaires. Completion of the surveys/questionnaires at the home will respect COVID-19 restrictions by diminishing time spent with the researcher. If the participant is not able to complete the surveys/questionnaires prior to their first scheduled visit, they will be given the opportunity to do at WinPO prior to the physical function evaluation. At the conclusion of the phone interview, the researcher will randomly assign participants to the control or exercise groups. Participants in both groups will then be scheduled for an in-person baseline visit and an in-person follow up visit at WinPO for data collection. The baseline visit will begin with review of expectations and the participant will sign informed consent. All information gathered at the phone interview will be reviewed for accuracy. If the participant has not answered the questionnaires/surveys prior to their baseline visit, they will be provided an iPad to complete the Medical and Demographic Questionnaire, Waterloo Footedness Questionnaire, and The Survey of Phantom Limb Awareness and Control. The baseline testing session will be entirely the same for participants of both groups. During this session, the researcher will assess participants based on their anthropometric variables (i.e. height, weight, body mass index), the morphology of the residual limb (i.e. length, circumference, and volume), prosthesis embodiment as measured by TAPES-R and PEmbS-LLA, and functional abilities as measured by targeted phantom motor execution and FSST, while recording their muscle activities and plantar foot loads. After completion of the baseline testing, the researcher will contact Dr. XXXX, to get information with respect to what surgical closure technique was performed for the participant.

There will be a three-week interval between the first and the second (last) testing sessions. Participants randomized to the control group will be encouraged to continue with their usual and customary exercise program. Participants in the exercise group will be instructed on performing a targeted phantom exercise program to be included in their usual and customary exercise program. At the second testing session, the same researcher will repeat all baseline measures. All data collection, including screening questionnaires and surveys of the study, will be performed through the secure REDCap platform (https://www.project-redcap.org/).

Experimental protocol

At the beginning of the baseline testing session, the researcher measures participants' weight and height using a scale and stadiometer; the body mass index will be calculated using REDCap programmed formula. Then the researcher will survey prosthetic embodiment by asking participants to fill out the revised version of the Trinity Amputation and Prosthesis Experience Scales (TAPES-R) and Prosthesis Embodiment Scale for Lower Limb Amputees (PEmbS-LLA), which are brief self-administered questionnaires to assess the adjustment and interaction of people with amputation to their prosthesis. Then, the researcher will ask participants to sit on a chair within parallel bars and remove their prosthesis. The researcher will examine the residual limb and places paper skin markers on specific landmarks over their residual limb (i.e. middle of the patella, tibial tuberosity, the distal end of the residual limb). Then, participants will stand securely inside parallel bars while the researcher captures the shape of their residual limb using a 3D scanner (OMEGA Scanner 3D, Willow Wood, Ohio, USA). The morphologic characteristics of the residual limb (i.e. length, circumference, and volume) will be recorded. If the participant finds it difficult to stand without the prosthesis, the scan will be performed in sitting and note in the assessment. The participant will then return to the sitting position and placement for the electromyography (EMG) electrodes (Trigno, Delsys Inc., Massachusetts, USA) will be determined on both the intact and amputated limbs based on the standard electrode placement guideline. However, for the amputated side, since the length of the muscles has changed, the location of the EMG electrodes needs to be determined specifically for each participant based on the new muscle bulk and optimal electrical signal activity. Electromyography of the tibialis anterior, medial and lateral heads of the gastrocnemius muscle will be recorded for both sides. Electrode locations will be marked and photographed to ensure consistency with subsequent testing session recordings. The skin will be cleaned using an alcohol pad and electrodes will be attached using paper tape.

Physical function assessments will be done by performing targeted phantom motor executions and four-square step test (FSST). Muscle activity will be recorded for the targeted phantom motor executions while participants are not wearing their prosthesis. However, the assessment of FSST needs participants to wear their prosthesis without EMG recording. The justification for this decision is preventing the risk of injury at the skin-prosthesis interface due to electrodes and wires. Foot plantar load will be assessed during targeted phantom motor executions and FSST. Participants will rest 2 minutes while sitting in a chair after each physical function assessment (4 minutes in total). This time will be used to instruct the participant in the testing protocol. Additional time will be provided if requested by the participant. After baseline testing is complete, EMG electrodes and sensors for the foot plantar load will be removed. Participants will then be informed of their randomization group that was determined earlier (before their baseline assessment) using the block and simple randomization techniques.

Collected data for the morphology of the residual limb, phantom phenomena, TAPES-R, PEmbS-LLA, physical function assessments, and muscle activities of the first session are baseline data and will be used for comparison to the results of the same measurements of the second session. After the first session, participants in the exercise group will be enrolled in the targeted phantom limb exercise program in addition to continuing with their usual and customary post-amputation care while participants of the control group will continue with their usual and customary post-amputation care. Participants in the exercise group will be trained on the targeted phantom limb exercise program and will have specific training sessions for three weeks at participants' homes via Zoom (https://zoom.us/). The researcher will make Zoom video calls to supervise participants and get assurance that all instructions are followed. After three weeks, participants of both groups will attend the test center for the second testing session. For both testing sessions, the same researcher captures the shape of the residual limb, surveys the phantom phenomena, TAPES-R, and PEmbS-LLA, assesses physical function, and conducts EMG investigations.

Data analysis and statistics

Different levels of comparisons will be done to explore the impact of the targeted phantom motor execution program on phantom phenomena and physical function of people with TTA. Before starting analyses, the normality of distribution of the quantitative variables will be assessed by the Shapiro-Wilk test. Since there are two groups of participants, whether participants are matched on their demographic and clinical characteristics will be explored using independent t-test and chi-square statistics. Based on our expectation of normality of distribution for the quantitative variables, if it is the case, parametric statistical tests will be used. An independent t-test will be used to compare quantitative variables within-subjects for the amputated and non-amputated sides. Similarly, Mann-Whitney test and Chi square test will be used respectively to compare ordinal and nominal categorical variables within-subjects for the amputated and non-amputated sides. An independent t-test will be analyzed to compare quantitative variables between the two groups. Similarly, Mann-Whitney test and Chi square test will be used respectively to compare ordinal and nominal categorical variables between the two groups.

Two-way analysis of variance can be used to compare the simultaneous effect of groups assignment and prosthesis embodiment on PLC. Similarly, Friedman's two-way analysis of variance will be used to compare the simultaneous effect of group assignment and surgical closure on PLC.

Spearman's correlation coefficients will be calculated to explore the potential relationship of phantom phenomena responses with participants' characteristics (i.e. age, weight, height, body mass index, length of the residual limb, the circumference of the residual limb, time after amputation, and years of prosthesis use). Moreover, the potential relationship of the phantom phenomena responses with participants' gender, type of surgery, and footedness will be explored by point-biserial correlation assessment. The potential

relationship of the phantom phenomena responses with participants' prosthesis type will be explored by partial eta square correlation assessment.

The linear regression analysis will be used to predict the effect of predictors / independent variables (i.e. clinical and demographic characteristics, and participants group) on outcomes / dependant variables (i.e. participants' physical function variables). Logistic regression analysis will be used to predict model of phantom phenomena based on participants' clinical and demographic characteristics, participants' group and physical function measures. Prior to regression analysis, regression assumptions including the normality, linearity, homoscedasticity (i.e. having equal variances in residuals of the predictors), and absence of multicollinearity (i.e. strong linear correlation between predictors as indicated by the average of the variance inflation factor (VIF) greater than 1) will be checked. For all statistical analyses, a p-value less than 0.05 will be considered statistically significant. The effect size and confidence intervals will be reported alongside other results to provide better judgment for the potential effects. All data will be analyzed offline using IBM SPSS software (Version 22.0, IBM Corp, New York, USA).