**“Cognitive-Motor Dual-Task Interference During Recovery from Unexpected Balance Loss in Individuals with Lower Limb Amputations Using Prostheses”**

**"הפרעה קוגניטיבית-מוטורית במהלך התאוששות מאובדן שיווי משקל בלתי צפוי בקרב אנשים עם קטיעה בגף תחתון הולכים עם פרוטזה"**

**Keywords:**

**Scientific Abstract:**

**1. Significance and Goal**

Dual-task performance in human subjects has received considerable interest in cognitive neuroscience, providing detailed insights into neural mechanisms underlying higher-order cognitive control. The concurrent performance of cognitive and motor tasks yields a different interference pattern. The pattern includes four major isolated changes: motor task facilitation, motor task interference, cognitive task facilitation, and cognitive task interference. There may also be combinations of changes or no changes1. Dual Task (DT) interference (DTi) occurs when two distinct simultaneous tasks deteriorate performance in one or both tasks. As a specific kind of DTi, cognitive-motor interference (CMi) occurs when the DT paradigm includes a motor task, such as reactive balance performance, and a cognitive task, such as cellular phone conversations or reading street signs. During DT performance, any modification from the reference single task (ST) condition in one or both subtasks is measured mostly as a percentage of change. This phenomenon is known as a DT cost (DTC)2. Whenever one or both performed STs change during DT condition, a CMi is likely present. Despite decades of research, our understanding of the neurobiological mechanisms underlying dual-task performance in individuals with lower limb loss using prostheses (LLPs) remains limited. Critical questions are still underexplored, such as whether LLPs respond effectively to unexpected balance loss while walking and performing a cognitive task, a DT condition.

Individuals with LLPs experience challenges in mobility3 due to loss of limb structures, peripheral afferent feedback, distorted somatosensory inputs from the amputated side, and efferent control4. These challenges include impaired postural stability5, increased energy expenditure while walking6, decreased gait speed7, gait asymmetries8, falls9,10, and increased likelihood of injurious falls11,12. Among the most common causes of falls are unexpected balance perturbations due to slips and trips while walking with an LLP13. The existing literature describes reactive balance responses triggered by unexpected perturbations during standing and walking in young adults14-17, older adults18-22, and stroke survivors23. However, there are fewer studies of these responses in adults with LLPs, despite being particularly vulnerable to falls and fall-related injuries. Also, in real-world situations, loss of balance may occur under DT conditions, leading to DTi and impairment of the balance reactive response. LLPs often report the need to “concentrate on every step” 24, indicating increased reliance on cognitive resources to compensate for diminished peripheral sensory feedback and motor control. Reduced confidence in balance and functional mobility further increases cognitive demands for movement and maintaining balance25. The heightened cognitive demand may reduce the availability of central nervous system (CNS) resources for concurrent tasks, such as maintaining balance, performing executive functions, and recovering from unexpected balance loss. More research is needed to understand how DTi impacts LLP users in such challenging scenarios. ***Lack of knowledge about LLP balance recovery presents a critical knowledge gap that must be addressed***. Competition may limit central processing resources if balance and cognitive tasks require cognitive resources26. If it occurs, competition will lead to DTi and impaired balance reactive response, impaired cognitive performance, or both27. Introducing walking perturbations during a concurrent cognitive task holds real-world relevance, necessitating dynamic balance adjustments to recover balance and prevent a fall. ***Investigating reactive balance responses to unexpected balance loss during walking in LLPs under DT conditions may discover mechanisms for balance control in this understudied at-risk clinical population.***

My team has developed the Balance Measure and Perturbation System (BaMPer), a motion platform designed to deliver varying levels of unexpected surface perturbations to simulate trips or slips during walking or standing. The power of our system is the ability to study balance responses under diverse conditions. Our data shows that, compared to older adult non-fallers, fallers initiate reactive stepping at a smaller perturbation amplitude (i.e., lower single-step thresholds), require multiple steps to recover balance, exhibit slower response times, take shorter step lengths, and have higher rates of simulated falls based on harness deployment17-19. We also find that stroke survivors have lower single- and multiple-step thresholds, shorter step lengths, and greater rates of laboratory-induced falls, especially toward the affected/paretic side23. Our results and critical need motivated our proposal to identify the mechanisms of balance control by LLPs when experiencing unexpected balance loss, particularly during DT. We will also investigate DTi to understand the availability of CNS resources in LLPs.

**1.1 Objectives and primary hypothesis of the proposal:** The primary goal of our proposal is to assess the impact of balance loss on LLPs.

**Objective 1. Impact of DTi on LLPs.** We will examine DTi under three scenarios: 1) reactive stepping performance when balance is lost unexpectedly during walking and standing, 2) voluntary proactive step performances, and 3) cognitive performance during reactive and proactive step performances in DT conditions. The results of LLPs will be compared to DTi in able-bodied, age-matched controls.

**Objective 2. Impact of DTi on different levels of amputation.** We will compare DTi between different levels of amputation, specifically unilateral transtibial amputation (TT) versus unilateral transfemoral amputation (TF).

Our objectives will address four significant hypotheses.

***Hypothesis 1.***LLP will have lower reactive step performance in DT compared to ST condition though higher DTi, relying primarily on their intact leg. LLP users will have significantly lower reactive step performance and higher DTi compared to able-bodied controls.

***Hypothesis 2.*** Compared to ST, LLPs, and able-bodied controls are likely to initiate and complete the proactive voluntary stepping slower in DT conditions. DTi during voluntary stepping will be significantly greater for LLPs than able-bodied controls.

***Hypothesis 3.***The cognitive DTi effect among LLPs will be greater during the voluntary step test compared to the reactive step test, and no DTi is anticipated in able-bodied controls.

***Hypothesis 4.*** CTFs will show a more impaired reactive and proactive stepping performance than TTs in ST and DT conditions. Additionally, TFs will exhibit greater DTi effects on cognitive performance during reactive and proactive stepping, indicating a stronger reliance on the ‘posture first’ strategy. The posture-first strategy is a prioritization mechanism observed in DT conditions where individuals maintain balance over other tasks. In such cases, the individual may shift attention away from the cognitive task and allocate more cognitive resources to maintain balance, ensuring stability and avoiding falls.

**2. Scientific Background**

**2.1. Epidemiological rationale for the proposal:** Falls are the 6th most common cause of death in older adults28 and the leading cause of injury-related visits to emergency rooms29. Annual falls and fall-related treatment costs were approximately $50 billion in 202028. Studies show that 50 - 60.2% of those living in the community fall at least once per year, and 33-36% experience multiple falls after completing a comprehensive rehabilitation program24,30,31. A systematic review of 12 studies reports that falls in LLPs are common, reporting 58% in the community years after the amputation9. Injurious falls are also common, ranging from 40-60% of falls9, with 19.3% requiring medical attention32. These statistics highlight the value of research into falls with the aim of prevention and rehabilitation. LLPs are at obvious risk because of their lower ability to react effectively during loss of balance and falls given their altered balance33, poor lower limb sense34, reduced muscle strength7, and increased gait impairments7,33-35. Many falls in LLPs occur due to unexpected balance loss while walking, affecting 48% of transtibial amputees and 80% of transfemoral amputees12. The consequences of these falls include limb fracture12,13, fear of falling24, lack of prosthesis use36, and subsequent social withdrawal37. The conflicts in Afghanistan, Iraq, and now in Israel add to the number of individuals with lower limb loss. ***Despite this knowledge, mechanisms of reactive balance control and information about the potential DTi and falling among LLPs are scarce.*** ***We will address this knowledge gap by investigating DTi mechanisms of balance control in LLPs in reactive and proactive voluntary movements as LLPs walk and engage simultaneously in a cognitive task emulating real-world situations.***

**2.2. Voluntary movements and balance control:** Due to the multi-link structure of the human body, any voluntary movement imposes a perturbation of balance. Our voluntary movements are accompanied by so-called Associated Postural Adjustments (APAs) to compensate for the perturbation. These “automatic” movements are smoothly incorporated into our movement repertoire to ensure accurate, harmonious motion38,39. Two parallel systems control movements: a voluntary element controlling the actual movement and a balance element that regulates the base of support provided by the feet and the center of mass (CoM), called APAs38,39. The interaction between voluntary movements and associated postural adjustments have been studied extensively during different movements, including rapid arm movements38 and leg stepping movements39-47. We conducted several studies examining voluntary stepping performance as a risk factor for falls43-48. We found significant dual-task interference (DTi) in voluntary step reaction time and step duration, particularly in older adults and stroke patient populations. Our research distinguished between young and old adults43,44, stroke survivors, and healthy controls48, identifying older adults with fall histories45, forecasting injurious falls46, and predicting future falls in older adults47.

**2.3. External perturbation and balance control:** During standing, unexpected external perturbations of balance trigger automatic postural responses to restore equilibria with a delay of about 100 ms. The responses are specific to the size, type, and direction of the imposed perturbation38-42. Minor perturbations at the feet trigger fix-of-support strategies, such as ankle-strategy or hip-strategy42. If the person cannot regain balance, a reactive step is initiated, indicating that fix-of-support strategies failed to recover balance40-42. The reactive step strategy is the most important postural response to prevent a fall directly38-42. These balance recovery responses have been studied extensively by our team in healthy young and old adults16-17, older adults with a history of falls 18-21, older adults responding unsuccessfully during the experiment resulting in falls20, and stroke survivors23. We find that older adult fallers and stroke survivors initiate reactive stepping at a smaller perturbation amplitude. They also display reduced step length, slower response and step times, and cannot control their CoM. Thus, they require multiple steps, more time to recover their balance, and a failure to regain equilibrium, resulting in a fall with harness deployment16-21,23. Cordo & Nashner38,39 suggested that the balance reactive response following external perturbation receives higher priority than a voluntary action, resulting in a faster reactive step than a “volitional” step. A reactive stepping often lacks the APA elements present in non-reactive voluntary stepping42. When present, these APAs appear to have little functional value during rapid reactive stepping. Lateral destabilization complicates the control of reactive stepping. The destabilization is coupled with decreased “crossover” steps and increased collisions between the swing and stance legs during lateral perturbations 23. This finding may be particularly relevant to the problem of balance loss and falls in LLPs lacking one limb and when stepping to recover balance.

In a meta-analysis of 54 studies (n=8.385)49 testing volitional versus reactive stepping to distinguish fallers from non-fallers, old adults showed that stepping performance was significantly worse in fallers compared to non-fallers (Cohen’s d 0.55, 95% CI 0.48-0.66, p<0.001, I 2 68%). This result was true for both volitional and reactive step tests. Twenty-two studies (n=3,503) were included in a diagnostic meta-analysis showing that step tests have moderate sensitivity (0.70, 95% CI 0.61-0.77), specificity (0.69, 95% CI 0.61-0.77), and area under the receiver operating characteristics curve (0.76, 95% CI 0.67-0.83) in discriminating fallers from non-fallers. ***We propose measuring both volitional and proactive reactive stepping as a primary outcome. Understanding the neural mechanisms involved in these parallel systems during DTi may lead to new diagnostic and therapeutic approaches and novel prosthetic limbs that detect imbalance, preventing LLP falls when cognitive attention is allocated elsewhere.***

**2.4. Responses to unexpected perturbation in LLPs:** An unexpected loss of balance during locomotor activities is one of the leading causes of falls50. Tripping and slipping during walking are the most common causes of falls, accounting for 59% of community-dwelling older adults51 and among LLPs10.These falls may pose a greater risk of injury in a lateral direction42, a factor that may be more pronounced in LLPs12,31. Our team and others worldwide have studied age-related deteriorations in balance recovery responses to unexpected perturbations in standing and walking14-22,43-47. LLPs face considerable challenges in maintaining balance due to disrupted motor and proprioceptive function, leading to a significant increase in fall risk, especially during walking10,12,31. Because slips and trips account for a significant proportion of falls, the ability to simulate them repeatedly and reliably and evoke a response in a laboratory is of critical clinical and scientific value. Examining reactive balance in LLPs under DT conditions is needed because there is little data evaluating responses to a loss of balance while walking. Olensk et al.52 exposed 14 LLPs and nine able-bodied controls to pelvis perturbations at the time of foot strike of the left or right leg. When outward-directed perturbations were delivered to the non-amputated leg, LLPs modulated their center of pressure and ground reaction force similarly to able-bodied controls. However, when perturbations were delivered during the prosthetic foot strike, LLPs utilized a stepping strategy and adjusted intact limb placement in the ensuing stance phase. This strategy resulted in a crossover step with a significantly larger displacement of the CoM. Sheehan et al. 53 found that LLPs experienced greater destabilization after exposure to lateral walking surface perturbations compared to able-bodied controls. Segal et al.54 studied the recovery responses of LLPs to an imposed error in mediolateral foot placement. When a prosthetic medial disturbance occurred, LLPs required five steps to regain undisturbed step width, whereas able-bodied controls accomplished this in two steps. Medial disturbances particularly challenged LLPs to the prosthetic limb, aligning with Olensk et al.52 and Sheehan et al.53 results.

Unfortunately, these well-designed studies were pilot investigations or lacked measures of reactive balance response while engaging in a concurrent cognitive-demanding task. Thus, previous studies failed to capture real-life situations52-54. Laboratory investigations of reactive stepping behavior in LLPs commonly involve a single task, enabling subjects to focus their cognitive attention on the upcoming motor task. In a real-life situation, the step commonly occurs under more complicated circumstances where attention is focused on reading street ads or talking, for example. ***The effect of an additional task on the balance reactive performance of LLPs is understudied, particularly with respect to balance reactive performance presenting a significant knowledge gap.*** Simultaneous cognitive and balance tasks may contribute to impaired balance and falls55 and provide an opportunity to study the neural mechanisms of balance control. A dual-task procedure was developed to estimate the level of automaticity and evaluate available CNS processing resources.

Most theories of cognitive function conclude that CNS processing resources are limited55, resulting in resource competition in performing one or more tasks. The competition leads to DTi and difficulty performing motor tasks55,56. If a reactive step is required to prevent a fall under attention-demanding circumstances, a delay in executing the reactive step may directly cause a fall and ensuing injuries. Four main cognitive-motor interforce patterns exist when performing concurrent motor and cognitive tasks1,58. 1) ***Motor-related cognitive interference***(motor task prioritized, cognitive performance deteriorates), posture first strategy, 2) ***Cognitive-related motor interference***(cognitive task prioritized, motor performance deteriorates), cognitive first strategy, 3) ***Mutual interference***(both tasks equally prioritized, performance on both deteriorates), and 4) ***Mutual* *facilitation***(both tasks equally prioritized, performance on both improves).

***Here, we ask whether an attention-demanding cognitive task will delay the execution of a reactive step and voluntary stepping, both motor tasks critical to maintaining and regaining balance during standing and walking. We propose to examine the effect of DTi on reactive stepping responses after unexpected perturbations of balance in walking and standing in a large group of LLPs.***

**2.5. Neural mechanisms of balance control during DT:** Several theoretical models may help explain the underlying neural mechanisms of DTi57-61.

**1.** ***The Capacity Sharing Model*** contends that processing capacity is finite, so performing more than one task concurrently requires capacity sharing, which impedes the performance of at least one task.

**2.** ***The Bottleneck Model*** proposes that different tasks may require similar information processing networks, resulting in a processing bottleneck. Due to concurring tasks, a secondary task’s performance is delayed, or a primary task’s performance is slowed.

**3. The *Crosstalk Model/Competition Model*** focuses on the type of information processedrather than the operations required for task performance. When two tasks require similar inputs, interference may occur; however, the opposite may occur. It may be easier to perform the tasks concurrently if they do not interfere with each other. There is no interference in the latter scenario, and improved performance may be observed under DT conditions.

**4.*****The U-shaped Model***states that when postural and cognitive tasks are performed simultaneously, postural performance may decline or improve depending on the cognitive load. **5. *The Task Prioritization Model*** contends that when a perceived postural threat is substantial, participants may use a posture-first strategy where postural performance is prioritized over cognitive performance. In this case, cognitive performance is expected to be reduced.

**2.6. Dual task in LLPs**: Interactions between cognition and posture indicate that people with impaired balance tend to increase their reliance on cognitive resources for motor control tasks compared to healthy controls27.The critical role of cognitive resources in postural functions has also been demonstrated in imaging studies62 and in kinematic research applying the DT methodology27,63. DT studies allow researchers to explore DTi, often referred to as DT costs or DT effects1,64,65. DT effects elucidate trade-offs between postural and cognitive tasks and task prioritization1,64,65, allowing the testing of interactions between cognitive CNS recourses and balance. LLPs lack direct sensory feedback from their peripheral components65 and perceive a greater need to concentrate while walking31. Thus, even experienced LLPs may require more CNS cognitive resources to control balance65,66. Most DT research involving LLPs has focused on DT effects on postural balance during standing and unperturbed gait. For example, concurrent serial arithmetic subtracting of three resulted in poorer gait performance for transtibial prosthesis users66. DT gait testing among TT prostheses users results in lower performance across several gait parameters, including reduced velocity, cadence, stride time, step length, and stance time. Additionally, participants spent less time on the prosthetic limb during single-limb support67,68. A systematic review reported that a cognitive task had a similar impact (DTi) on walking performance in LLPs and able-bodied controls65. However, walking was slower with wider steps and more asymmetry in LLPs as they adopted a conservative walking strategy. This strategy may reduce the need to concentrate on walking and contribute to notable gait deviations. ***We argue that there are weaknesses in previous studies, which do not include quantitative objective measures of balance reactive function performance in DT (DTi) among LLPs******.***

**3. Preliminary Data**

**3.1. Balance Measure and Perturbation System (BaMPer System):** We developed the BaMPer System, patent number PCT/IB2010/052079. It can provide controlled, unpredictable perturbations while walking in all horizontal directions.

**3.2. Reactive stepping responses while standing and walking:** We exposed 84 older adults (79.3±5.2 years) to perturbations in standing that were gradually increased to trigger a recovery stepping response19. Though a small effect, subjects took significantly longer to initiate a recovery step and substantially longer to complete the recovery step as the magnitude of perturbation increased19. We found a significant increase in the total spectral power of lower-limb muscles during the first three seconds after perturbation, indicating the possible use of fast twitch muscle fibers during balance recovery. Fallers also had significantly lower single- and multiple-step threshold levels than non-faller older adults. Recurrent fallers exhibited a significant delay in step initiation duration, longer step duration, greater CoM displacement, and an extended period for complete balance recovery compared with non-fallers18. We examined the unsuccessful recovery trials where older adults failed to recover and fell into the harness system20. The first reactive step response was significantly slower, and the crossover step was used during the unsuccessful recovery trials.

**3.3. Reactive stepping responses in stroke survivors:** Like LLPs, persons with stroke are at increased risk of falls. We exposed 30 subacute people with stroke and 15 controls to perturbations while standing. The controls demonstrated significantly lower fall single- and multiple-step thresholds, whereas the 25 people with stroke fell into the harness system23.

**3.4. Reactive stepping responses while performing a dual task:** Twenty older adults17 and 13 younger adults21 performed five test conditions: (1) cognitive task while sitting, (2) perturbed standing with no concurrent cognitive task, (3) perturbed standing with a concurrent cognitive task, (4) perturbed walking with no perturbations, and (5) perturbed walking with a concurrent cognitive task. Kinematic reactive balance parameters were similar for both age groups in ST and DT conditions. This result indicates that when the postural threat is substantial, such as unexpected balance loss during walking, balance recovery reactions are automatic and unaffected. However, we only found a reduction in cognitive DT performance in older adults, indicating a posture-first strategy.

**3.5. Reactive stepping response in LLP users:** In an unpublished pilot study, we subjected nine LLPs and ten able-bodied controls to unexpected perturbations, as described in this proposal. The single-step threshold was half the length in LLPs (4.5 cm) compared to controls (9 cm). Fall events, defined by load cell sensors detecting 30% or more body weight suspended by the safety harness, occurred only in LLPs (four of the nine). The results indicate that our protocol is feasible and effective for investigating reactive balance mechanisms in LLPs.

**4. Summary of Innovations and Impact**

The BaMPer System offers a reliable assessment of recovery stepping responses in a safe and controlled manner. Its unpredictable, multidirectional perturbations closely mimic real-world falls. Experimentally, we will trigger reactive balance responses during walking and standing, providing high unpredictability under ST and DT conditions and effectively simulating real-life falls. The rationale for our proposal is three-fold. **We will 1) Examine the DTi effects on balance reactive stepping, 2) Examine DTi effects on balance proactive stepping, and 3) Explore whether cognitive performance accuracy is affected by DT conditions to examine between task trade-offs.** This approach will permit the dissection of relative changes between ST and DT conditions, the DT costs (i.e., DTC/DTi), and if the spatiotemporal characteristics of the reactive step and proactive voluntary step are affected similarly by concurrent cognitive load. These innovations will test two hypotheses.

**Hypothesis 1.** Recovery responses will be prioritized over cognitive performance in a posture-first strategy when the postural threat is substantial and similar to real-life balance loss.

**Hypothesis 2**. DTi will result in a trade-off favoring postural performance. Namely, no postural DTi will be found, whereas cognitive DTi will decline DT cognitive performance accuracy. Based on our previous data, we predict that voluntary proactive stepping will be significantly reduced in DT versus ST conditions, validating hypothesis 2.

**Hypothesis 3.** Per the task prioritization model, the interference effect of an unexpected balance loss will result in decreased cognitive performance during DT.

**Hypothesis 4.** DTi will manifest as a trade-off favoring postural performance. Namely, no postural DTi will be found, whereas cognitive DTi will result in a relative decline in DT cognitive accuracy in reactive and even proactive voluntary step performance. Voluntary steps require more cognitive attention, planning, and execution of APAs than reactive stepping, a reflex-like automatic response.

**5. Comprehensive Description of Methodology and Plan of Operation**

**5.1. Study Design and Methods**: The proposal encompasses three key aims. We will compare 48 LLPs (20 TT and 20 in TF) and 20 able-bodied controls in BaMPer testing.Recruitment will follow our previous perturbation studies15-21,43-47. Forty-eight LLPs will be screened by Dr. Treger MD (Head of the Rehabilitation Department, Soroka Medical Center). Our criteria for inclusion of LLPs will be unilateral 20 TT and 20 TF amputation, 20 years or older, use of a prosthesis for at least a year, and ability to walk independently without an assistive device for five consecutive minutes. We will include 20 able-bodied age-matched controls as “Gold standard” controls. We will exclude participants with one or more of the following: amputation of a second limb, dysvascular amputation, wound, severe arthritis, joint replacement of the contralateral leg, neurological or cardiovascular condition limiting gait, COPD, uncontrolled blood pressure, blindness or severe vision problems, vestibular deficit, severe cognitive problems (Mini-mental test score < 24), or exceed 120kg, the weight limit of the safety harness. Prior to the experiment, participants will receive a study explanation and sign informed consent forms approved by local ethics committees.

**5.2. Assessment protocol:** We will assess test subjects and evaluate the data using a set of detailed criteria.

***5.2.1. Reactive step responses*:** We will expose participants to unannounced surface translation perturbations during standing and walking at six progressive magnitudes to anterior/posterior and lateral surface translation, triggering a recovery stepping response. Handrails are not mounted on the perturbation system to permit unconstrained arm movements. The participants will be secured with a full trunk safety harness designed to allow for free motion but prevent ground contact and injury in case of a fall. We will study the following conditions: 1) a cognitive task while sitting, 2) no cognitive task during perturbed standing (ST standing), 3) a concurrent cognitive task during perturbed standing (DT standing), 4) no cognitive task during perturbed walking (ST walking), and 5) a concurrent cognitive task during perturbed walking (DT walking). During the standing trials, each perturbation magnitude will include four directions in a randomized order, posterior/anterior/lateral-right surface translation representing four directions x six perturbation magnitudes totaling 24 perturbed trials. The perturbation characteristics will be based on platform acceleration, velocity, and displacement. The intervals between perturbations will be 30-50 seconds. To maintain consistency during the standing trials and to increase the challenge, we will instruct participants to stand with their feet together (toes & heels touching) and hands close to the sides of their bodies. They will then be instructed to “try to avoid a fall.”

In the walking trials, we will choose the habitual self-selected walking speed for each participant wearing their own comfortable walking shoes. The characteristics of the perturbation magnitudes will be like the standing trials. At each perturbation level, there will be right/left perturbations in two directions by six magnitudes for a total of12 trials. We will randomize the intervals between the perturbations and their direction in each trial. We will instruct the participants to “walk naturally and try to avoid a fall.” During the ST and DT walking trials, participants will have 60 seconds to adjust to treadmill walking before the first perturbation occurs. This period will allow us to examine the effects of walking without perturbations on the performance of the concurrent cognitive task during the DT walking trials. **Note:** Only the right/left perturbation trials will be analyzed because of the difficulty of identifying the step threshold during the forward/backward walking trials and the time in which the participants initiated their reactive recovery responses. The cognitive task used for DTs will be serial subtractions by seven, which is related to executive function components17,21. We will give three different starting numbers for each test condition to avoid a learning effect: sitting DT, perturbed standing DT, and perturbed walking DT. We will instruct participants to count continuously until a stop cue is given. ***Cognitive performance will be assessed as the counting accuracy under each test condition.*** We will first calculate the numbers for each condition, divided by the duration of the test condition. We will then calculate the total numbers(correct numbers + errors) for each test condition, divided by the test duration. ***Finally, we will calculate the cognitive performance ratio between the two means as follows:***

***Dual-task costs (DTC)*** will be calculated to elucidate trade-offs between postural and cognitive tasks and task prioritization1,64, allowing examination of interactions between cognitive recourses and postural functions. We will calculate the DTCs separately for each task condition during the standing and walking trials according to the traditional formula:

***5.2.2. Analysis of reactive stepping performance:*** Kinematic data will be recorded using a 3D Vicon motion capture system (Vicon Motion Systems, Oxford, UK). Using an internal direct linear transformation algorithm, we will map views from the 16 Vicon cameras onto a 3D coordinate system (Vicon Nexus system software, version 2.5). In addition, two reflective markers on the perturbation system will track the initiation of BaMPer motion.The time windows of interest will be approximately 2 seconds pre-perturbation and 5 seconds post-perturbation to examine the subject recovery response behavior.We will analyze data exported from both motion analysis systems using our custom Matlab code (Math Works Inc., Cambridge, MA, USA) developed previously. We will analyze their responses to ongoing events using the following model.

***First*** ***Reactive Step Initiation Time*** (ms) is defined at the first deviation of the marker placed on the perturbation system to foot-lift off the ground. ***Step Length*** is calculated as the Euclidean distance (cm) that the ankle markers displaced from foot-lift to foot contact on the ground, completing the recovery step. The Reactive Step Time is calculated as the time (ms) from BaMPer movement to foot contact on the ground, completing the recovery step. The ***Margins of Stability*** **(MoS)** will be calculated using the equation:

**.**

xCoM indicates the CoM position, vCoM indicates the CoM velocity, *g* represents gravitational acceleration, and *l* represents the leg length calculated from markers attached to the greater trochanter of the femur. BoS represents the area beneath a person encircled by the points of contact of their feet with the supporting surface and BoSpos is the lateral edge of BoS, calculated using the lateral malleolus marker position.

In the standing trials, there are three parameters of reactive balance. 1. **Single-step threshold** is the minimum perturbation magnitude eliciting a recovery step. 2. **Multiple-step threshold** is the minimum perturbation magnitude eliciting more than a single recovery step. 3. **Fall threshold** is the minimum perturbation magnitude to recover and fall into the harness system. We previously found15 found excellent inter-observer reliability of balance recovery responses for step thresholds and the kinematics parameters of stepping (ICC = 0.98 & 0.97; p<0.001).

***5.2.3. Proactive stepping performance:*** *For the* ***Step Voluntary Execution Test,*** we will instruct participants to stand on a force plate, then take six steps forward and six steps backward as quickly as possible following a tester’s light tap on the foot. The step should be at least 0.3 m long, as marked by a line. Subjects will then take three steps with the affected leg and three with the unaffected leg in a randomized order under ST and DT conditions while performing a concurrent Stroop test, to a total of 24 proactive steps. We will collect four parameters: 1) initiation time, 2) preparation time (anticipatory postural adjustment time), 3) swing time duration, and 4) foot contact time from the tap cue to the foot placed on the ground completing the step. Each of the phases of proactive stepping is dominated, although not exclusively, by different physiological processes. The division allows us to understand the specific effects of the ST and DT tests. The duration of the step initiation phase depends mainly on peripheral sensory detection and the afferent nerve conduction time, followed by the central neural processing and efferent nerve conduction time. During the preparatory phase, anticipatory postural adjustments (APA) are executed, and the step is initiated. Finally, the swing phase incorporates motor execution of the task when the leg is lifted and moved to the target location. The duration of the swing phase is mainly dependent on neuromotor mechanisms related to the build-up of muscle force and power to move the leg. We have shown that the step execution parameters are sensitive to age43,44, retrospective falls45, prospective falls47, and injurious falls46.

**5.3. Clinical outcome measures:** We will measure clinical outcomes in three ways.

***5.3.1. The Amputee Mobility Predictor (AMP):*** The AMP is a performance-based tool designed to measure the functional status of LLPs. We will ask LLPs to complete static and dynamic tasks by rising from a chair or standing on one leg with progressive difficulty. Higher scores indicate better functional ability. The AMP scores will be used to characterize participant Medicare Functional Classification Level (MFCL) using the five-level code modifiers (K0-K4)[74]. We will classify fallers and non-fallers using the AMP cut-off score69.

***3.3.2 Fall Efficacy Scale-International (FES-I):*** The FES-I is designed to assess potential fall concerns. FES-I uses 16 questions describing social and physical activities, both indoors and outdoors. Each question is ranked on a scale of 1-4 (1= Not at all concerned, 4= Very concerned), where a higher score reflects increased apprehension about falls70.

***3.3.3. Self-reported fall:*** We will ask LLP users whether they have fallen in the last year using the definition “a loss of balance where the body landed on the ground or floor.”

**5.4. Statistical analysis and sample size estimation:** We will perform statistical analyses using Predictive Analytics Software (PASW v 26.0; Somers, NY). The statistical significance for all hypotheses will be set *a priori* at *p* < 0.05. ***To test our 1st hypothesis comparing ST versus DT performance in the two LLP user groups and able-bodied controls, we will apply General Linear Models (GLM) for each outcome parameter of balance control, specifically balance recovery parameters.*** For example, GLM for three groups (20 TT LLPs, 20 TF LLPs, and 20 able-bodied controls) by four motor task conditions (perturbed standing ST, Perturbed standing DT, perturbed walking ST, and Perturbed walking DT conditions) for each reactive balance parameter. ***To test the 2nd hypothesis concerning the level of amputation and task (ST/DT) on voluntary step parameters, we will apply General Linear Models (GLM) for each outcome parameter of proactive balance.*** We will also apply GLM to evaluate the overall interference effect of the concurrent attention-demanding task (dual task normalized to a single task within each group) between the three groups. ***To test the 3rd hypothesis, we will compare cognitive performance between task conditions in four ways***. 1. A cognitive task while sitting, 2. A concurrent cognitive task during perturbed standing, 3. A concurrent cognitive task during unperturbed walking, 4. A concurrent cognitive task during perturbed walking.

**5.5. Sample size:** We calculated our sample size based on the outcomes of our pilot experiment related to the reactive postural mechanism as single-step thresholds. The single-step thresholds of able-bodied controls were 9±3cm versus 4.5±3.3cm for LLPs. Using net reduction values (4.5) in combination with the initial variance estimates (SD = 3), we will need to study at least 13 subjects of each experimental group and 13 controls to be able to reject the null hypothesis. We hypothesize that stepping kinematics during the reactive balance tests will be similar between ST and DT conditions, as these responses are automatic and reflex-like, with no significant DTi effects expected. As a result, we do not base our sample size on the kinematics of reactive stepping. Our second hypothesis posits that voluntary, proactive step performance will be significantly reduced for DT compared to STs, consistent with the task prioritization model for proactive stepping. Since this will be the first study to measure proactive stepping under ST and DT conditions for LLPs, our sample size estimation is based on findings from our previous study, which reported a 298ms ± 332 ms difference in voluntary step time between ST and DT conditions in older adults. Based on these calculations, a minimum of 20 subjects is required, using a two-sided estimate at a significance level of 0.05 and 80% power.

**6. Risk Analysis**

**1. Exclusion of dysvascular LLPs*.*** Dysvascular LLPs have lower balance and a higher prevalence of comorbidities. Thus, we cannot generalize the results to this population, which should investigated in the future

**2.** **Completion of reactive balance protocol*.*** Some LLPs may be unable to complete the perturbed walking task condition. In this case, we will use the perturbed standing trials to measure balance recovery reactions

**3.** **Serial subtractions by seven**. While this cognitive task may not reflect cognition performance in real-life situations, it is a known and reliable measure of executive function