**1. Significance and Goal**

Dual task (DT) 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 several interference patterns. The interference patterns include 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. 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 response if balance is lost unexpectedly, and concurrently performing 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 usually measured 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 DT 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 concurrently performing a cognitive task, a DT condition.

Individuals with LLPs experience challenges in mobility3 due to loss of limb structures, loss of 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 an 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 function presents a critical knowledge gap that must be addressed***. Competition may limit central processing resources if both 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 population.***

My team has developed the Balance Measure and Perturbation System (BaMPer), a motion platform designed to deliver varying levels of unexpected surface perturbations during walking or standing to simulate trips or slips. The power of our system is the ability to study balance reactive 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 simulated falls, especially toward the paretic side23. Our results and a 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 Research objectives and primary hypothesis of the proposal:**

The current project is based on the following four hypotheses.

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

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

***Hypothesis 3.***If the reactive balance response is an automatic reflex-like response, the cognitive DTi effect among LLPs will be greater during the voluntary step test compared to the reactive step test, and no cognitive DTi effect is anticipated in able-bodied controls.

***Hypothesis 4.*** Unilateral transfemoral amputation (TF) subjects will show more impaired reactive and proactive stepping performance than unilateral transtibial amputation (TT) subjects 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 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.

**To investigate these hypotheses further, our Specific Objectives are:**

**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, minimizing the influence of age-related factors.

**Objective 2. Impact of DTi on different levels of amputation.** We will compare DTi between different levels of amputation, specifically TT vs.TF, the latter requiring more complex motor control of knee and ankle joints.

**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 LLPs fall at least once per year, and 33-36% experience multiple falls24,30,31. A systematic review of 12 studies reports that falls in LLPs are common, reporting 58% 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 in understanding the mechanisms of reactive balance and falls with the aim of prevention and rehabilitation. LLPs are at obvious risk of falls because of their lower ability to react effectively during loss of balance 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 people with amputation and 80% of transfemoral people with amputation12. 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 movement imposes a perturbation of balance. Our voluntary movements are accompanied by so-called anticipatory 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), which is 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 six studies examining voluntary stepping performance as a risk factor for falls43-48. We found significant DT interference (DTi) in voluntary step reaction time and step duration, particularly in older adults and stroke patient populations. Our research distinguished young vs. old adults43,44, identifying older adults with fall histories45, forecasting injurious falls46, and predicting future falls in older adults47 and stroke survivors vs. healthy controls48.

**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 vs. old adults16-17, older adults with a history of falls18-21, older adults responding unsuccessfully during the experiment resulting in falls20, and stroke survivors23. We find that older adult fallers, as well as stroke survivors, initiate reactive stepping at a smaller perturbation amplitude compared to healthy age-matched controls. 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 even a failure to regain equilibrium, resulting in a fall with harness deployment16-21,23. Cordo & Nashner38,39 hypothesized 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. 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. In addition, we found that lateral destabilization in response to lateral perturbation 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 perturbations23. 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 stepping and reactive stepping to distinguish fallers from non-fallers, old adults (age 65 years old and over) 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 volitional proactive stepping and reactive stepping as a primary outcome. Both steps represent different mechanisms of balance control. A proactive stepping response is an anticipatory postural adjustment to maintain balance before a voluntary movement begins. In contrast, a reactive step response is a compensatory postural adjustment to maintain balance after an unexpected perturbation event. Understanding the neural mechanisms involved in these different control systems during DTi may lead to a better understanding of the underlying balance control mechanisms among LLPs. This knowledge may result in new diagnostic and therapeutic approaches and novel prosthetic limbs that detect imbalance, preventing LLP falls even 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 in this population. 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 prosthetic 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 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. Previous studies failed to capture real-life situations and were unable to investigate the DTi effects52-54. Laboratory investigations of reactive stepping behavior in LLPs commonly involve an ST, enabling subjects to focus their cognitive attention on the upcoming motor task. A DT procedure was developed to estimate the level of automaticity and evaluate available CNS processing resources. ***The effect of an additional cognitive task on the balance reactive performance of LLPs is understudied, particularly with respect to balance reactive performance, which presents 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 CNS processing resources and balance control***.

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 tasks or cognitive 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. ***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.*** 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).

**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 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 and may require more CNS cognitive resources to control their balance65,66, enhancing the need to investigate DTi effects. 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 support of the gait cycle67,68. 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******. Here, we propose to test these recovery strategies under DT.***

**2.7. Previous relevant work by the investigator:**

***2.7.1. 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.

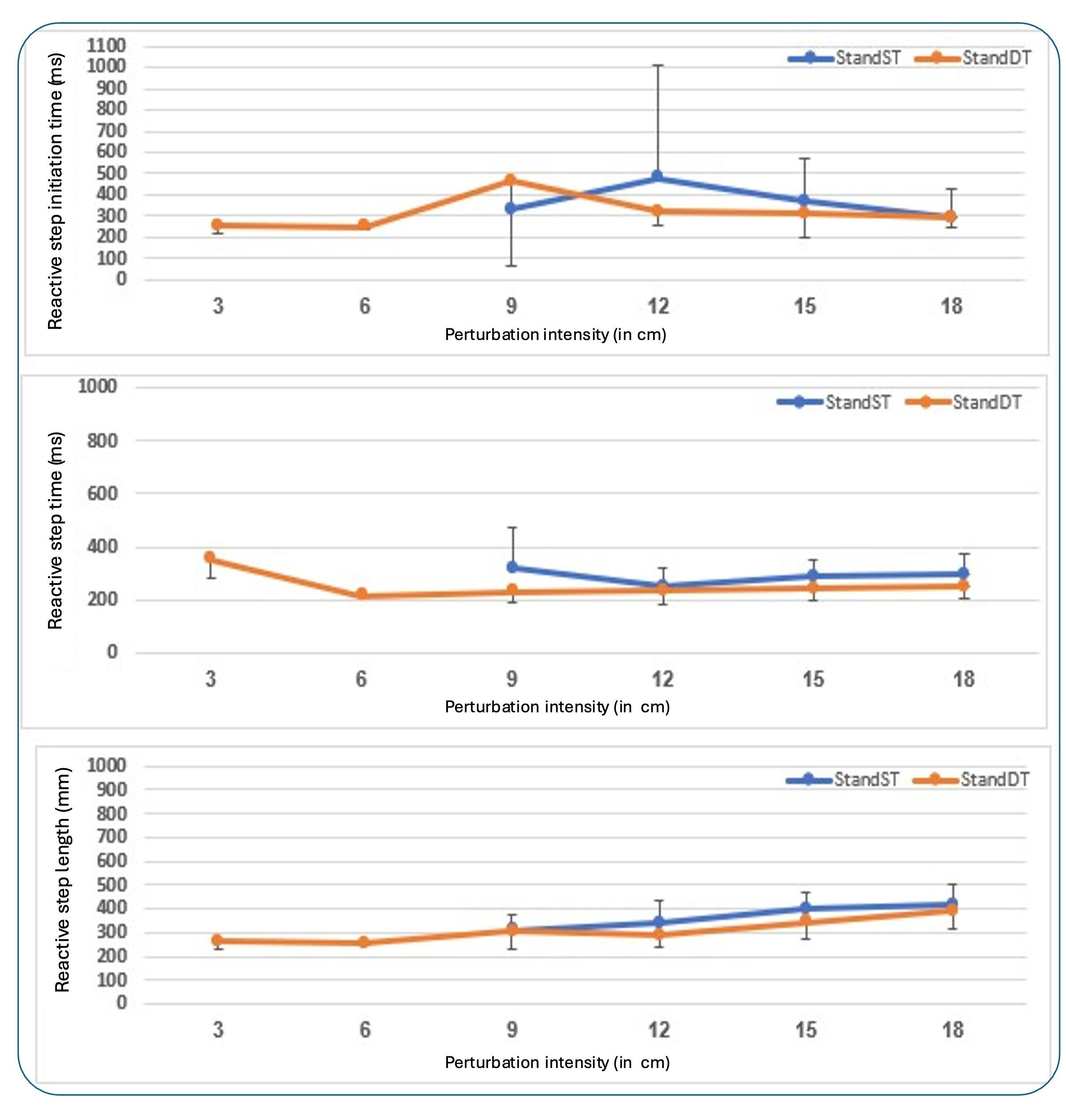
***2.7.2. Reactive stepping responses while standing and walking:*** We exposed 84 older adults (79.3±5.2 years) to perturbations in standing that were gradually increased to trigger a recovery stepping response19. Though a small effect, subjects took significantly longer to initiate a recovery step and significantly 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.

***2.7.3. Reactive stepping responses in stroke survivors:*** Like LLPs, persons with stroke are at increased risk of falling. We exposed 30 subacute people with stroke and 15 controls to perturbation while standing. The controls demonstrated significantly lower fall single- and multiple-step thresholds, and 25 people with stroke fell into the harness system during the experiment23.

***2.7.4. Reactive stepping responses while performing a dual task:*** Twenty older adults17 and 13 younger adults (aged 20-30 years old)21 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 reflex-like responses, and the motor tasks are unaffected by DT. However, we found a reduction in cognitive DT performance in older adults only, indicating a posture-first strategy mechanism was used to recover balance during DT.

**3. Preliminary Data**

**Reactive stepping response in LLPs:** In an unpublished pilot research study, we subjected nine LLPs and ten able-bodied controls to unexpected perturbations in standing, as described in this proposal (see section **5.2 Experimental protocols**). 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). In addition, we found that the single-step threshold among LLPs was lower in the DT condition compared to the ST condition during perturbations. Also, LLPs predominantly performed reactive steps with the affected limb. Interestingly, when the first reactive steps were triggered, the kinematics in the ST condition were like those in the DT condition **(Figure 1)**. The observation indicates that when the kinematics of the first reactive response follows a sudden loss of balance, the initial reactive step is likely an automatic reflex-like response. The results indicate that our protocol is feasible and appropriate for investigating reactive balance mechanisms in LLPs.



**Figure 1(A-C):** Kinematics of the first reactive step performance in standing among nine LLPs under Single task (StandST, blue) and DT (StandDT, orange) conditions. **Note**: No reactive steps were performed in ST condition in low intensities (intensity 3 and 6). **Abbreviations**: ms, milliseconds; cm, centimeters.

**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. This innovative use of our step-recovery system will be used for three innovative experimental approaches. **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 as well as proactive voluntary step performance are affected similarly by concurrent cognitive load.

These innovations will test the following 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 reactive performance. Namely, no postural DTi will be found, whereas cognitive DTi will decline DT cognitive performance accuracy. However, based on our previous data, I predict that voluntary proactive stepping will be significantly reduced in DT versus ST conditions.

**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 in balance reactive performance, whereas cognitive DTi will result in a relative decline in DT cognitive accuracy in reactive. In proactive voluntary step performance, a significant DTi will be found as the voluntary steps require more cognitive attention, planning, and execution of APAs than reactive stepping, a reflex-like automatic response.

**5. Detailed Description of Proposed Research**

**5.1. Study Design**: The proposal encompasses three key aims. We will compare 40 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 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 amputations, 20 years or older, use of a prosthesis for at least a year, and ability to walk independently without an assistive device for at least five consecutive minutes. We will include 20 able-bodied age-matched 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 Helsinki ethics committees.

**5.2. Experimental 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 while standing and walking at six progressive magnitudes to anterior/posterior and lateral surface translation, triggering a recovery stepping response. Handrails will not be 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 **(Table 1)**.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, the participant will choose his/her habitual self-selected walking speed while wearing 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 in ST and 12 trials in DT conditions. 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 at 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 during the 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:***

***DT costs (DTC)*** also defined as DTi, 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 intervals of interest will be approximately two seconds pre- and five 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 subject responses to ongoing events using the following calculations: ***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 swing phase Time*** is calculated as the time 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. In addition, we will calculate the ***Margins of Stability*** **(MoS)** 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, which is calculated using the lateral malleolus marker position.

In the standing trials, there are three parameters of reactive balance: 1. **Single-step threshold** is defined as the perturbation magnitude eliciting a single recovery step. 2. **Multiple-step threshold** is defined as the perturbation magnitude eliciting more than a single recovery step to recover balance. 3. **Fall threshold** is defined as the perturbation magnitude in which the participants are unable to recover balance and fall into the harness system based on harness deployment. We found excellent ICC of balance recovery responses for step thresholds and kinematics of stepping (0.97-0.98; p<0.001)15.

***5.2.3. Proactive stepping performance:*** *For the* ***Step Voluntary Execution Test,*** we will instruct participants to stand on a Kistler 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. During the ST trials, LLPs will then take six steps with the prosthetic leg and six with the non-prosthetic leg in a randomized order of 12 under ST conditions and 12 under DT conditions while performing a concurrent Stroop test for a total of 24 proactive steps. We will collect five parameters: 1) initiation time, 2) preparation time (anticipatory postural adjustment time), 3) swing time duration, 4) time from tap to foot-lift off the ground, and 5) 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 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, APA is 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. The division allows us to understand the specific effects of the ST and DT conditions. We have shown that the step execution parameters are sensitive to age43,44, retrospective falls in older adults45, prospective falls47, and even 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. The LLPs are asked 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)69.

***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 LLPs whether they have fallen in the last year, defined as a loss of balance in which the body lands 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 reactive step performance in the two LLP user groups and able-bodied controls, we will apply general linear models (GLM) for each outcome parameter of balance recovery.*** For example, GLM for three groups (20 TTs, 20 TFs, 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 comparing ST vs. DT of proactive voluntary stepping performance in the two LLP user groups and able-bodied controls,*** *we will apply General Linear Models (GLM) for each test condition and each outcome parameter of voluntary stepping****.***
* ***To test the 3rd hypothesis concerning interference effects, we will compare cognitive performance between task conditions during a loss of balance 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, and 4. A concurrent cognitive task during perturbed walking. We will also ***compare cognitive performance between task conditions during voluntary step performance (ST vs. DT) between the groups.*** We will apply GLM to evaluate the overall interference effect (DTi) of the concurrent attention-demanding DT normalized to a ST within each group).
* ***To test the 4th hypothesis concerning the level of amputation (TTs vs. TFs) and task (ST/DT) on reactive step and voluntary step parameters,*** *we will apply GLM for each outcome parameter of proactive balance.*

**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. Based on our pilot experiment, we hypothesize that in these cases, when the reactive step is triggered, the kinematics of the first reactive step will be similar between ST and DT conditions. 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 the first reactive step. Our second hypothesis posits that voluntary, proactive step performance will be significantly reduced for DT compared to STs. This prediction is consistent with the task prioritization model for proactive stepping and our previous investigations demonstrating a dramatic effect of a concurrent cognitive task on balance performance among old43-47, stroke survivor48, and even young subjects43,44. Because our proposal is the first study to measure proactive LLP stepping under ST and DT conditions, our sample size estimation is based on findings from our previous study43, which reported a 298ms difference in voluntary step time between ST and DT conditions in older adults with SD = 332ms. Based on these results, at least 20 subjects are required in each group, using a two-sided estimate at a significance level of 0.05 and 80% power.

**6. Expected Results and Pitfalls**

**1) Completion of reactive balance protocol**. To our knowledge, this proposal will be the first laboratory-based study to detect and investigate DTi effects on LLPs during unexpected balance loss while walking. We recognize that the five-minute perturbed walking protocol may be too difficult for some TFs. Thus, we added the alternative five-minute perturbed standing experimental protocol, allowing relatively more balance-impaired TFs to complete the perturbation testing protocol.

**2) Inclusion of both TT and TF LLP users**. Because TT LLP users have better balance and mobility than TF users, the inclusion of both amputation levels may interfere with our ability to find differences in fall recovery stepping responses. To reduce this possible limitation, we calculated the sample size needed and will recruit an equal number of participants in each group *(i.e., 50%* *TT and 50% TF LLP users)*.

**3) Exclusion of LLP users with amputation due to dysvascular disease*.*** People with dysvascular amputation have reduced balance and mobility due to a higher prevalence of comorbidities and decreased physical function. Thus, we cannot generalize the results to this specific population. Future studies should investigate this specific population.

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

Our results of this proposal will leverage future grant applications, particularly for our long-term goals of 1) prospectively investigating the ability of the BaMPer testing to predict falls in LLPs and 2) developing a prosthetic leg that can effectively react to unexpected balance loss. We will present the results of this project at national and international meetings and through peer-reviewed journals. We predict that the proposal will result in at least four peer-reviewed articles focusing on neuroscience as well as rehabilitation and prevention.

**7. Lab Resources and Facilities**

The Motion Analysis and Rehabilitation Laboratory, directed by **Prof. Itshak Melzer,** focuses on increasing knowledge of balance control, falls, and injury prevention in older adults. The following equipment will be available in the Lab: 3D Vicon system 16-infrared cameras, a 3D ZED2 camera, an eight-channel Myo-EMG system, two Kistler Force plates systems, and the BaMPer perturbation system.