**"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"**

**1. Significance and Goal**

The study of dual-task performance in human subjects has received considerable interest in cognitive neuroscience because it can provide detailed insights into the neural mechanisms underlying higher-order cognitive control. The concurrent performance of a cognitive and motor task yields to a different pattern of interference. This includes four major isolated changes (motor task facilitation, motor task interference, cognitive task facilitation, and cognitive task interference), or the possible combinations of these observations, as well as no changes at all1. Dual Task (DT) interference (DTi) occurs when the simultaneous performance of two different tasks results in the deterioration in one or both task performances. As a specific kind of DTi, the cognitive-motor interference (CMi) occurs when the DT paradigm includes a motor task (i.g., reactive balance performance) and a cognitive task (i.g., cellular phone conversations or reading street signs). During the 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 is also known as a DT cost (DTC)2. Whenever one or both of the performed ST(s) will change in a DT condition, a CMi will likely be 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, such as whether LLPs respond effectively to unexpected balance loss during walking and concurrently performing cognitive task, i.e., DT conditions, are still underexplored.

LLPs experience several 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 more likely to experience injurious falls11,12. Among the most common causes of falls are balance unexpected perturbations, i.e., slips and trips, that occur while walking with a LLP13. While the existing literature describes reactive balance responses triggered by unexpected perturbations during standing and walking in young adults14-17, older adults18-22 and in stroke survivors23, less effort has been made to study these responses in LLPs, even though they are particularly vulnerable to falls and fall-related injuries. Also, in real-world situations, loss of balance may occur during a concurrent performance of two tasks (i.e., DT conditions) which may lead to DTi and impairs balance reactive response. LLPs often report the need to "concentrate on every step"24, which suggests increased reliance on cognitive resources to compensate for the diminished peripheral sensory feedback and motor control. Reduced balance confidence and functional mobility further increase the cognitive demands required for movement and maintaining balance25. This heightened cognitive demand may reduce the availability of central nervous system (CNS) resources for other concurrent tasks, such as maintaining balance, performing executive functions and recover from unexpected balance loss. More research is needed to understand how DTi impacts LLPs in such challenging scenario. ***Investigating reactive balance responses to unexpected balance loss during walking in LLPs in DT conditions will help us to better understand mechanisms of balance control in this understudied at-risk clinical population.***If both balance and cognitive tasks require cognitive resources, resource competition may occur due to limited central processing resources26. If resource competition occurs, it will lead to DTi and impaired balance reactive response, or impaired cognitive performance, or both27. Introducing perturbations in walking, during a concurrent performance of a cognitive task, holds real-world relevance, necessitating dynamic balance adjustments to recover one’s balance and prevent a fall.

My team has developed the Balance Measure & Perturbation System (BaMPer), a motion platform designed to deliver varying levels of unexpected surface perturbations that simulate trips or slips during walking or standing. This system enables us to study balance responses under diverse conditions. Our data show 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 a higher rate of “falls” (i.e., harness deployment)17-19. We also found that stroke survivors have lower single- and multiple-step thresholds, shorter step lengths and higher laboratory-induced rates of “falls,” especially toward the affected/paretic side23. This data motivates a study aimed at identifying the mechanisms of balance control used by LLPs when they experience unexpected balance loss, particularly during DT. The study will measure DTi, which occurs when two different tasks are performed simultaneously, to better understand the availability of CNS resources in LLPs.

**1.1 Objective and primary hypothesis of the proposal**

The primary objective of this study is to assess the impact of DTi in LLPs during 1) reactive stepping performance when balance is lost unexpectedly during walking and during 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 effects in able-bodied, age-matched controls. A secondary objective is to compare DTi between different levels of amputation, specifically unilateral transtibial amputation (TT) versus unilateral transfemoral amputation (TF).

***Hypothesis 1:***LLPs are expected to have a lower reactive step performance in DT compared to ST condition i.e., higher DTi, relying primarily on their intact leg. LLPs expected to have a significantly lower reactive step performance & higher DTi compared to able-bodied controls.

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

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

In addition, compared to TTs, TFs are likely to show a more impaired reactive and proactive stepping performance in both ST and DT conditions. Additionally, TFs are expected to exhibit greater DTi effects on cognitive performance during both reactive and proactive stepping, indicating a stronger reliance on the 'posture first' strategy. The posture-first strategy refers to a prioritization mechanism commonly observed in DT conditions in which individuals focus on maintaining balance over other tasks. In such cases, the individual may shift attention away from the cognitive task and allocate more cognitive resources toward maintaining balance, ensuring stability to avoid falls.

**2. Scientific Background**

**2.1. Epidemiological rationale for the proposal:** Falls is the 6th most common cause of death in older adults28 and the leading cause of injury-related visits to emergency rooms29. Annual falls and falls-related treatment costs are approximately $50 billion in the 2020 year28. Studies show that 50%-60.2% LLPs who are living in the community fall at least once each year, with 33%-36% experiencing multiple falls after completing a comprehensive rehabilitation program24,30,31. A systematic review of 12 studies reported that falls in LLPs were a common occurrence, reporting 58% in the community years after the amputation9. Injurious falls also were common, with an occurrence ranging from 40%-60% of falls9, 19.3% require medical attention32. This highlights the potential for valuable insights into fall research with the aim of prevention, and rehabilitation. LLPs are at obvious risk for 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 and Iraq and now in Israel have added to the growing 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 still scarce. Consequently, ***we aimed to investigate DTi mechanisms of balance control in LLPs in both reactive and proactive voluntary movements while LLPs Walk and simultaneously engage in a cognitive task that emulates real-world situations.***

**2.2. Voluntary Movements and Balance Control:** Due to the multi-link structure of the human body where one segment is connected to another, any voluntary movement imposes a perturbation of balance. To compensate for this perturbation our voluntary movements are accompanied by so-called Associated Postural Adjustments (APAs). These “automatic” movements are smoothly incorporated into our movement repertoire to ensure accurate harmonious motion38,39. It was concluded that there are two parallel systems in the control of voluntary movements, one for the intended voluntary element of the actual movement and one for maintaining balance, regulating 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 since been studied extensively during different movements including arm rapid movements38 leg stepping movements39-47. We conducted several studies examining voluntary stepping performance as a risk factor for falls43-48. We found a significant dual-task interference (DTi) on voluntary step reaction time and step duration, particularly in older adults and stroke patient populations. Our research allowed us to distinguish between young and older adults43,44, stroke survivors and healthy controls48, identify older adults with a history of falls45, forecast injurious falls46 and predict future falls in older adults47.

**2.3. External Perturbation and balance control:** Unexpected external perturbations of balance in standing, trigger automatic postural responses with a delay of about 100 ms, which act to restore equilibrium. The responses are specific to the size, type and direction of the imposed perturbation38-42. Minor perturbations at the feet trigger what is known as a fix-of-support strategies e.g. “ankle-strategy”, or “hip-strategy”42. If the person cannot regain balance a reactive step will be initiated indicating that the fix-of-support strategies have failed to recover balance40-42. The reactive step strategy is the most important postural response that can directly prevent a fall38-42. These types of balance recovery responses to unexpected balance loss have been studied extensively by our team in healthy young and old adults16-17 and in older adults with a history of falls 18-21, older adults who responded unsuccessfully during the experiment, resulting in falls20 and in stroke survivors23. We found that older adult fallers (and stroke survivors) initiate reactive stepping at a smaller perturbation amplitude, reduction in step length; exhibit slower response and step times, unable to control the centre of mass, require multiple steps, thus more time to recover their balance, and failure to recover equilibrium and fall, i.e., harness deployment16-21,23. It was suggested that the balance reactive response following an external perturbation receives a higher priority than a voluntary action Cordo & Nashner38,39 resulting a faster reactive step compared to "volitional" step. A reactive stepping reaction often lack the APA elements that are invariably present in non-reactive voluntary stepping42. Even when present, these APAs appear to have little functional value during rapid reactive step. Lateral destabilization complicates the control of reactive stepping, this is coupled with a decreased likelihood of taking a “crossover” step, an increased frequency of collisions between the swing and stance legs during lateral perturbations 23, a finding that may be particularly relevant to the problem of losing balance and falls in LLPs due to the lack of one limb and when stepping to recover balance.

A meta-analysis49 of 54 studies (n= 8,385) that examined the type of step test (e.g. volitional vs. reactive stepping) that is required 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 was the case for both volitional and reactive step tests. Twenty-two studies (n=3,503) were included in a diagnostic meta-analysis that showed 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. ***In the present study, both, volitional, proactive and reactive stepping are selected as the primary outcome measures. An increased understanding of the neural mechanisms involved in these two parallel systems in the control of voluntary movements reactive and proactive stepping and the DTi, could lead to the development of new diagnostic and therapeutic approaches, as well as the design of novel prosthetic limbs capable of detecting imbalance and preventing falls in LLPs also in situations where the 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 main 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.Age-related deteriorations in balance recovery responses to unexpected perturbations have been studied extensively world-wide and by our team 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 slip and trip-related falls account for a significant proportion of falls, the ability to simulate a slip and trip and evoke a corresponding slip and trip-like response in a laboratory setting is of both clinical and scientific value repeatedly and reliably. Examination of reactive balance in LLPs, especially in DT conditions is needed as few data exist evaluating responses to a loss of balance while walking. Olensk et al.52 exposed 14 LLPs and 9 able-bodied controls to perturbations delivered to the pelvis at the time of foot strike of either the left or right leg. When outward-directed perturbations were delivered to the non-amputated leg, LLPs could modulate the center of pressure and ground reaction force similarly to able-bodied controls. However, when perturbations were delivered at the time of prosthetic foot strike, LLPs utilized a stepping strategy and adjusted placement of the intact limb in the ensuing stance phase to make a cross-over step, with a significantly larger displacement of the center of mass (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. LLPs were particularly challenged by medial disturbances to the prosthetic limb, which aligns with Olensk et al.52 and Sheehan et al.53. Unfortunately, even these well-designed studies have either been of a pilot nature or have not included measures of reactive balance response while engaging in a concurrent cognitive attention-demanding task, thus failing to capture true real-life situations52-54. Laboratory based studies of reactive stepping behavior in LLPs have commonly been single task in nature, i.e. subjects can focus their cognitive attention on performing the upcoming motor task. In a real-life situation, however, the requirement to step commonly occurs under more complicated circumstances and cognitive attention is focused elsewhere, e.g. on reading street ads or talk. The effect on balance reactive performance during conducting an additional distracting task in LLPs has been little studied, in particular with respect to a balance reactive performance. Simultaneous performance of cognitive and balance tasks has been suggested as a potential contributor to impaired balance and falls55 as well as provide ability to study 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 on cognitive function conclude that the available CNS processing resources are limited55.As a result, resource competition may occur during the performance of more than one task, leading to task interference (DTi) and difficulty in performing motor tasks55,56. If a reactive step is required to prevent a fall under attention-demanding circumstances, a delay in execution of reactive step may be the direct cause of a fall and ensuing injuries. In the current study we asked the question whether an attention-demanding cognitive task would delay the execution of a reactive step we will also examine voluntary stepping performance, both motor tasks of critical importance to the maintenance and regain of balance in standing and walking. ***In the proposed study, we examine in interreference effect of a concurrent attention-demanding cognitive task (DTi) on reactive stepping responses after unexpected perturbation of balance in a larger group of LLPs in walking and standing.*** There are main 4 cognitive-motor interforce patterns 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 have been proposed that may help explain underlying mechanisms of our future findings57-61: 1) The ***capacity sharing Model*** contends that processing capacity is finite, performing more than one task concurrently requires capacity sharing, which impedes performance of at least one task; 2) ***The bottleneck Model*** proposes that if different tasks require similar information processing networks and cannot be processed simultaneously, there will be a processing bottleneck. Due to concurring tasks, a secondary task's performance is delayed and/or a primary task's performance is slowed; 3) The ***cross-talk Model/competition model*** rather than focusing on the operations required for task performance, the cross-talk theory/competition model focuses on the type of information processed. When two tasks require similar inputs, interference may occur; however, the opposite may also occur; that is, it may be easier to perform such tasks concurrently if they do not interfere with each other. There is no interference in the latter scenario and performance improvement may even be observed under DT condition; 4) the ***U-shaped model****,* according to the model, when postural and cognitive tasks are performed simultaneously, postural performance can decline or improve depending on the level of cognitive load; 5) the ***task prioritization model*** contends that when perceived postural threat is substantial, participants may prioritize postural performance over the cognitive performance (i.e., posture first strategy). In this case, cognitive performance is expected to be reduced.

**2.6. Dual task in LLPs**: Studies examining interactions between cognitive and postural functions indicate that people with impaired balance function tend to increase reliance on cognitive resources for motor control tasks compared to healthy controls27.The critical role of cognitive resources in postural functions has been also 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, allow testing of interactions between cognitive CNS recourses and balance functions. Since LLPs experience loss of direct sensory feedback from their peripheral components65, and studies indicate that LLPs perceive a greater need to concentrate while walking31, even experienced LLPs may require more CNS cognitive resources to control balance65,66. Most of the DT research involving LLPs to date has focused on the effects of DT on postural balance during standing and unperturbed gait. For example, a concurrent serial arithmetic subtracting of 3 resulted in poorer gait performance for transtibial prosthesis users66. DT gait testing among TT prostheses users, resulted in lower performance across several gait parameters, including reduced velocity, cadence, stride time, step length, and stance time. Additionally, the participants were spending 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 both 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 but also contributed to notable gait deviations. ***We argue that there are weaknesses in the previous studies is that they have not included quantitative objective measures of balance reactive function performance in DT (DTi) among LLPs******.***

**2.7. Preliminary data**

**2.7.1. Balance Measure & Perturbation System (BaMPer System):** We developed the BaMPer system, patent number: PCT/IB2010/052079. The BaMPer 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. It took a small yet significantly longer time to initiate a recovery step and a significantly longer time to complete the recovery step as the magnitude of perturbation increased19. We also found that there is a significant increase in the total spectral power of lower-limb muscles during the first three seconds after perturbation, suggesting the use of fast twitch muscle fibres during balance recovery. We found that compared to non-fallers older adults, fallers had significantly lower single- and multiple-step threshold levels. 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 also examined the unsuccessful recovery trials where older adults failed to recover and fell into the harness system during the experiment20. The first reactive step response was significantly slower, and 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 falls. We exposed 30 subacute people with stroke and 15 controls to perturbations while standing. They demonstrated significantly lower fall single and multiple step thresholds, 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 youngs21 performed the following 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 (4) perturbed walking with a concurrent cognitive task. In both age groups, kinematic reactive balance parameters were similar in ST and DT conditions, suggesting that in situations where the postural threat is substantial, such as unexpected balance loss during walking, balance recovery reactions are automatic and were unaffected, however, we found a reduction in cognitive DT performance in older adults only, suggesting posture first strategy.

**2.7.5. Reactive Stepping response in LLP users:**In our pilot study (unpublished), 9 LLPs and 10 able-bodied controls were subjected to unexpected perturbations as proposed in the proposed study. The single-step threshold was twice as low in LLPs (4.5 cm) compared to controls (9 cm). “Fall” events (load cell sensors detected 30% or more body weight suspended by the safety harness) occurred in LLPs only (4 of the 9). The data suggest the protocol is feasible for LLPs and likely effective for investigating reactive balance mechanisms.

**2.8. Summary of innovations and impact**

The BaMPer system offers a reliable way to assess recovery stepping responses in a safe and controlled manner. Its unpredictable, multidirectional perturbations closely mimic real-world falls. In our experimental setup, we will trigger reactive balance responses during walking and standing, providing high unpredictability in both ST and DT conditions, simulating real-life falls more effectively. The rationale for the proposed study is to: 1) examine the DTi effects on **balance reactive stepping** abilities; 2) examine the DTi effects on **balance proactive stepping** abilities; 3) explore whether **cognitive performance accuracy** is affected by DT conditions. This will help to examine between task trade-offs. Hence, to further explore relative change between ST and DT conditions, and examine the DT costs (i.e., DTC/DTi). This will help examine whether the spatiotemporal characteristics of the reactive step and proactive voluntary step are similarly affected by concurrent cognitive load. We first hypothesize that recovery responses would be prioritized over cognitive performance (i.e., posture-first strategy) as the postural threat is substantial and very similar to real-life situations of balance loss. We hypothesize that DTi would manifest a trade-off in favour of the postural performance, that is, no postural DTi would be found while cognitive DTi would manifest a decline in DT cognitive performance accuracy. Based on our previous data our second hypothesis is that voluntary proactive step performance will be significantly reduced in DT condition vs. ST condition. In accordance with the task prioritization model, our third hypothesis is that due to the interference effect of an unexpected balance loss, cognitive performance will be affected in DT. We hypothesize that DTi would manifest a trade-off in favour of the postural performance, that is, no postural DTi would be found while cognitive DTi would manifest a relative decline in DT cognitive performance accuracy) in both reactive and even more during proactive voluntary step performance as it requires more cognitive attention, more planning executing APAs, than reactive stepping which is a more reflex-like automatic response.

**3. Comprehensive description of the methodology and plan of operation**

**3.1. Study Design**

The proposed study will be encompassing three key aims. It involves comparing 48 LLP users (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). Inclusion criteria for LLPs will be unilateral 20 TT and 20 TF amputation; 20 years or older; use of a prosthesis for at least a year; able to walk independently without an assistive device for 5 consecutive minutes. We will include 20 able-bodied age matched controls; they will serve as “Gold standard” controls. We will exclude participants who have an amputation of a second limb, dysvascular amputation; wound, severe arthritis, or joint replacement of the contralateral leg, neurological or cardiovascular condition that limits gait, COPD, uncontrolled blood pressure, blindness and/or severe vision problems, vestibular deficit, severe cognitive problems (Mini-mental test score < 24), are over 120kg (overweight this is an excess of the weight limit of the safety harness). Prior to the experiment, all participants will receive a study explanation and sign respective informed consent forms approved by local ethics committees.

**3.2. Assessment protocol**

**3.2.1. Reactive step responses:** As per our studies, participants will be exposed to unannounced surface translation perturbations during standing and walking at six progressive magnitudes to anterior/posterior and lateral surface translations during standing and walking to trigger a recovery stepping response. Handrails are not mounted on the perturbation system, so that arm movements will be unconstrained. 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. The following conditions will be studied: (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 perturbations in four directions in a randomized order: posterior/anterior/lateral-right surface translation for a total of 24 perturbed trials (i.e., 4 directions × 6 perturbation magnitudes). In the proposed study, the perturbation characteristics will be based on platform acceleration, velocity, and displacement. The intervals between perturbations will be 30-50 sec. To maintain consistency during the standing trials and to increase the challenge, the participants will stand with their feet together (toes & heels touching), hands close to the sides of their body, and will be instructed to “try to avoid a fall.”

In the walking trials, habitual self-selected walking speed will be chosen for each participant. Participants will walk while wearing their 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 for a total of12 perturbed trials (i.e., 2 directions × 6 perturbation magnitudes). The intervals between the perturbations and the perturbation direction will be randomized in each trial. The participants will be instructed 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 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 in DT conditions is serial subtractions by seven, which is known to be related to executive function components17,21. To avoid a learning effect, three different “starting numbers will be given in each test condition (i.e., sitting DT, perturbed standing DT, perturbed walking DT). Participants will be instructed to continuously count until a stop cue is given.**Cognitive performance** will express the counting accuracy under each test condition (i.e., sitting DT, perturbed standing DT, unperturbed walking DT, and perturbed walking DT).We will first calculate the correct numbers counted in each condition, divided by the duration of the test condition. We then calculate the total numbers counted(i.e., correct numbers + errors) in each test condition, divided by the duration of the test condition. Finally, we’ll calculate the **cognitive performance ratio** between the two means as follows:

$$Response accuracy \left(\%\right)= \frac{Total correct answers}{Total numbers counted} X100$$

**Dual-task costs (DTC)** will be calculated to elucidate trade-offs between postural and cognitive tasks as well as task prioritization1,64 and thus allow examination of interactions between cognitive recourses and postural functions. The DTCs will be calculated separately for each task condition during the standing trials and walking trials according to the traditional formula: $\frac{\left(DT-ST\right)}{ST}\*100=DTC\left(\%\right)$

**3.2.2. Analysis of Reactive stepping performance**

The kinematic data will be recorded using a 3D Vicon motion capture system (Vicon Motion Systems, Oxford, UK). Views from the 16 Vicon cameras will be mapped onto a 3D coordinate system by the computer (Vicon Nexus system software, version 2.5) using an internal direct linear transformation algorithm. In addition, two reflective markers on the perturbation system track the initiation of BaMPer motion.The time windows of interest will be approximately 2 seconds pre-perturbation and about 5 seconds post-perturbation to examine the recovery response behavior of the subjects.Data exported from both motion analysis systems will be analyzed using our custom code written in Matlab (Math Works Inc.; Cambridge, MA, USA) developed for our previous studies. We will analyze their responses to ongoing events: ***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; ***First*** ***Step Length*** is calculated as the Euclidean distance in cm that the ankle markers displaced from foot-lift to foot contact on the ground completing the recovery step. ***First*** Reactive ***Step Time*** (ms) is calculated as the time from BaMPer movement to foot contact on the ground, completing the recovery step. The ***Margins of Stability*** **(MoS)** will be calculated using the equation:$MoS = (xCoM+{vCoM}/{\sqrt{{g}/{l}}}-BoS\_{pos})$**.** Here, the xCoM indicates the CoM position, and vCoM indicates the CoM velocity. The *g* represents gravitational acceleration, and *l* represents the leg length calculated using the markers attached to the greater trochanter of the femur. The BoS represents the area beneath a person encircled by the points of contact that the person’s feet make with the supporting surface, and BoSpos is the lateral edge of BoS, which is calculated using the lateral malleolus marker’s position.

In the standing trials, the observational parameters of reactive balance are 1) **Single-step threshold**, defined as the minimal perturbation magnitude that elicits a recovery stepping response; 2) **Multiple-step threshold**, defined as the minimal perturbation magnitude that elicits more than a single recovery stepping response; 3) **Fall threshold**, defined as the minimal perturbation magnitude that the participant fell to recover and fell into the harness system. In a previous study15 we found excellent inter-observer reliability of balance recovery responses for step thresholds and for the kinematics parameters of stepping ((ICC = 0.98 & 0.97; p<0.001).

**3.2.3. Proactive stepping performance - *Step Voluntary Execution Test:*** Participants will be instructed to stand on a force plate and take 6 steps forward and 6 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, 3 with the affected leg and 3 with the unaffected leg in a randomized order and in two task conditions: ST and in DT condition while performing a concurrent stroop test, to a total of 24 proactive steps. Several parameters will be collected: 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 are dominated, although not exclusively, by different physiological processes, this division allows us to better understand the specific effects of the ST and DT test conditions. The duration of the step initiation phase is mainly dependent on peripheral sensory detection and afferent nerve conduction time followed by central neural processing and efferent nerve conduction time. During the preparatory phase, anticipatory postural adjustments (APA) are executed, and the actual step is initiated. Finally, the swing phase incorporates the actual 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 step execution parameters have been shown by Melzer’s team to be sensitive to age43,44, retrospective falls45, prospective falls47, and injurious falls46.

**3.3. Clinical outcome measures**

***3.3.1. The Amputee Mobility Predictor (AMP):*** A performance-based assessment tool designed to measure the functional status of LLP users. They are asked to complete static and dynamic tasks (e.g., rising from a chair, 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 5-level code modifiers (K0-K4)[74]. The AMP cut-off score will classify fallers and non-fallers69.

***3.3.2 Fall Efficacy Scale-International (FES-I):*** A tool designed to assess concern regarding potential falls. 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 potential falls70.

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

**3.4. Statistical analysis and sample size estimation**

Statistical analyses will be performed using Predictive Analytics Software (PASW v 26.0; Somers, NY). Statistical significance for all hypotheses was set *a priori* at *p* < 0.05. **To test our 1st hypothesis**, comparing ST vs. 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 3 groups (20 TT LLPs, 20 TF LLPs and 20 able-bodied controls) X 4 motor task conditions (Perturbed standing ST and Perturbed standing DT, Perturbed walking ST and Perturbed walking DT conditions) for each reactive balance parameter. **To test the 2nd hypothesis,** the effects of level of amputation, task condition (ST/DT) on voluntary step test parameters we will apply General Linear Models (GLM) for each outcome parameter of proactive balance. We will also apply General Linear Models (GLM) to evaluate the overall interference effect of the concurrent attention-demanding task (dual task normalized to single task within each group) between the three groups. **To test the 3rd hypothesis,** we will compare cognitive performance between task conditions (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.

**3.5. Sample size:** we calculated the sample size based on outcome measures in our pilot that are related to the reactive postural mechanism (e.g., single-step thresholds). The single-step thresholds of able-bodied were 9±3cm vs. 4.5±3.3cm in LLPs. Using net reduction values (4.5) in combination with the initial variance estimates (SD of 3), we found that we need to study at least 13 subjects of each experimental group and 13 control subjects to be able to reject the null hypothesis. We hypothesize that the kinematics of stepping 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 did not base our sample size calculation on kinematics of reactive stepping. Our second hypothesis posits that voluntary proactive step performance will be significantly reduced in DT compared to ST conditions, 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±332ms 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.

**4. Risk analysis: 1) *Exclusion of dysvascular LLPs.*** dysvascular LLPs have lower balance, a higher prevalence of comorbidities. Thus, we cannot generalize the results to this population that should investigated in the future; 2) **Completion of reactive balance protocol*.*** It is possible that some of the LLPs won’t be able 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 it may not be a cognitive performance in real-life situations, this cognitive task is a known and reliable measure of executive function