**סמנים פיזיולוגיים לחוסן דינמי בקרב קשישים**

**Physiological Markers of Dynamic Resilience in Elderly Populations**

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**Significance and Goal**

The doubling of human life expectancy over the last century, from a global average of 35 years in 1900 to more than 70 years today[1], is a remarkable success story. This progress is evident in the increasing number of centenarians and supercentenarians, demonstrating humans can lead fulfilling lives for extended periods. Despite numerous health challenges throughout their long lives, these exceptional individuals exhibit striking resilience, exemplified by the ability to recover from significant stress events such as falls, diseases, infections, surgeries, or psychosocial stresses. Such resilience results from complex physiological systems incorporating baseline reserve, stress detection, and adaptation capabilities. Resilience also results from the speed and appropriateness of responses, including the ability to heal and recover, that enable the body to return to homeostasis following disruptions. Present assessments of dynamic resiliencearebased on subjective questionnairesthat may not objectively quantify the physiological responses accompanying stress events. **Objectively understanding the physiological mechanisms of dynamic resilience among older adults offers valuable insights into promoting healthy aging that may enhance human life expectancy**.

We can now harness our ability to trigger a real-life physiological stress event in the laboratory to discern dynamic prognostic markers associated with human resilience. Novel in vitro platforms allow us to construct human-derived systems of physiological relevance to evaluate human responses to laboratory stress events. The PI, Prof. Melzer, has developed a novel motion platform, the **B**alance **M**easure & **Per**turbation System (BaMPer), which can simulate unexpected loss of balance while the participant walks on a mechatronic system. Our data shows that after exposure to unexpected balance loss, the sympathetic nervous system is triggered even among young adults. Interestingly, some participants show high resilience to this event, whereas others show lower resilience (see **Preliminary Results). This data provides the basis for this ISF proposal, where our overall goal is to understand the physiological mechanisms of dynamic resilience among older adults by simulating a stress event.** Through our Objectives and Specific Aims, we will use the BaMPer system to develop makers for resilience and construct a multivariate model that will result in a dynamic resilience prediction score (*dRePS)*.

**The primary goal of this proposal is to identify personalized biomarkers indicative of physiological resilience**. A major output of the proposal will be identifying and validating causal measures and models of dynamic resilience that are sufficiently predictive to inform clinical decision-making and intervention. Notably, a 25% reduction in the progression to frailty among individuals over age 65 would safeguard the well-being of up to 87 million people globally [2]. This aspiration is entirely within the realm of possibility, as frailty has demonstrated potential for arrest and even reversal28.

Our Objectives below will provide the foundation for enhancing the capacity for measurement, modeling, and testing critical for innovative methodologies to enhance holistic well-being. We will address the following key questions: How can we effectively gauge and identify individuals at the highest risk of experiencing health deterioration following a stress-inducing event? That is, can we identify individuals with "fragile dynamic resilience"? What factors underlie the variability of dynamic resilience, where some individuals maintain fragile dynamic resilience, and some do not? In particular, what underlies instances where individuals exhibit frailty levels by static measurements disparate from dynamic resilience responses? Our proposal is structured around two Objectives.

**Objective 1:** We will expose 70-year-old adults (n=60) to the laboratory-induced stress event of unexpected loss of balance while walking. ***Hypothesis 1: Thirty percent of older adults will show fragile dynamic resilience, namely less adaptive physiologically dynamic resilient behavior.***

**Objective 2:** We will develop a multivariate model to construct a dRePSbased on markers from our proposed investigation. ***Hypothesis 2: A "signature of resilience" after a laboratory-induced stress event can be identified that will be particularly valuable for the elderly population***. The prediction score will be a weighted summary of identified biomarkers determined by the model coefficients, including sympathetic nervous system and cortisol responses, functional markers, and brain markers. ***We further hypothesize that markers from discovery cohorts will demonstrate that resilience markers associate significantly with (p<0.05) and predict immediate recovery from a laboratory-induced stress event of unexpected perturbation and a loss of balance during walking.***

**Scientific Background**

**Epidemiological rationale for the proposal:** As we age, most individuals experience a decline in baseline reserves and a gradual deterioration of biological maintenance systems, culminating in a significant burden of age-related long-term conditions, often manifesting as multimorbidity. Approximately 50% of adults aged over 65 suffer from at least two age-related long-term conditions, a figure that escalates to nearly 80% among those over 80[3]. Furthermore, our capacity to cope with stress diminishes with age, rendering us susceptible to sudden and severe health deterioration upon encountering acute illness or injury. In clinical terminology, this diminished resilience or vulnerability is termed "frailty" and afflicts around half of the population aged 65 and older who exhibit frailty or are at risk of progressing to frailty[4]. Consequently, our shortened duration of good health, known as our "health span", is considerably outstripped by our overall lifespan. This fact underscores the profound consequences of reduced resilience with age[5].

Escalating global aging has brought frailty to the forefront as a pressing and burgeoning health issue. Notably, the proportion of adults over 65 has reached 11.8% in Israel, over 16% in Singapore, 17% in the US, 19% in the UK, and a substantial 30% in Japan[6]. Projections indicate that by 2050, over 1.5 billion individuals will surpass age 65, with the oldest over 85 expected to triple[7]. Alarming statistics reveal that more than a quarter of adults over 65 experience falls annually, with frailty contributing significantly. In the US, hip fractures from falls lead to the hospitalization of approximately 285,000 individuals and, tragically, 32,000 fatalities[8]. Similarly, frailty accounts for more than 70% of unplanned hospital admissions and contributes to 20% of hospital bed occupancy in the UK[9]. Frailty also notably amplifies the risk of death by 8- to 10-fold following emergency hospital admissions[10]. For frail patients, surgical interventions carry heightened risks, leading to prolonged hospital stays, adverse outcomes, loss of independence, discharge to long-term care, or even death[8,11]. The detrimental impact of frailty and age-related health issues on older individuals, their families, caregivers, and societies is unsustainable[12], necessitating urgent attention and proactive interventions to address this global health crisis.

**Resilience as a new framework to promote healthy aging:** Knowledge concerning biological aging is well-established[13]. The knowledge is underscored by growing acknowledgment that age-related ailments, multimorbidity, and frailty result from inherent cellular and molecular alterations instigated by aging[14]. Focusing on a resilience perspective is a novel framework for comprehending health dynamics during aging. The framework reinstates static and dynamic resilience to ameliorate the erosion of homeostatic equilibrium, which precludes frailty and age-associated multimorbidity. Anti-aging therapies have attracted multi-billion-dollar investments[15] as researchers worldwide develop interventions to enhance health during aging. There is a pressing need to establish quantitative, predictive, and robust metrics to expedite this scientific endeavor to elucidate the mechanisms that maintain the physiological steady state. Markers of dynamic resilience are imperative, particularly in response to stress-inducing events. Frailty scores and indices offer reasonably accurate predictions regarding aggregate statistical outcomes of stress events, such as recovery, debilitation, or mortality. However, the efficacy of such tools to predict individual outcomes is limited[16]. Even precise techniques like methylation or immune-based aging clocks[17] primarily gauge an individual's physiological state at a specific moment without assessing dynamic resilience, namely the capacity to adapt to and recuperate from stresses.

Our critical objective is to identify and quantify parameters governing resilience and overall health within complex biological systems. In conjunction, we seek to understand the intricate biological processes and factors underpinning the preservation and restoration of homeostasis. The parameters may encompass enhancements in immune responses, improved energy regulation, or robust stress-coping mechanisms, as demonstrated among centenarians[18]. Rigorously validated models incorporating the identified parameters and spanning scales from molecular-level to tissue-level examinations, and ultimately the entire human body, can be instrumental tools for unraveling causal mechanisms. Moreover, these tools may aid in the identification of individuals at risk of health deterioration from stress-event-induced factors. Significantly, such models may expedite clinical investigations of interventions to sustain or reinstate resilience. Accurate prediction of individual dynamic resilience and the ability of older adults to return to homeostasis after disruption, health challenges, and stress events is scarce and insufficient. **We propose to identify predictors and causes of human resilience by utilizing a unique design integrating data from physiological and biological systems, physical performance measures, brain imaging, and emotional markers to investigate dynamic resilience, which is the ability to "bounce back" after a significant stress event.**

**Dynamic resilience and frailty:** Dynamic resilience may be crucial in preventing or delaying the onset of frailty in older adults[19]. Low dynamic resilience is a major contributor to frailty and disability, which may increase the risk of death in older adults[19]. Dynamic resilience and frailty are two related concepts when considering the well-being of older adults. Notably, frailty increased from 4.2% of community-dwelling older adults in 2015 to 6.7% in 2020. Over 40% of older adults were considered pre-frail, with higher rates among females[20]. Frailty is characterized by a decline in multiple physiological systems, including physical, cognitive, nutritional, and social functioning[21,22]. Frailty also impairs homeostatic balance, conferring extreme stress vulnerability, resulting in negative health risks for mortality and disability[23]. Dynamic resilience, by contrast, focuses on the ability to adapt to challenges and changing circumstances and thrive[23]. It emphasizes the capacity to recover, learn, and respond effectively to new situations and stressful events[23]. While frailty and dynamic resilience appear contradictory, they can coexist and influence each other. There are five key points regarding their relationship.

1. **Prevention and delay of frailty**: Frailty can be prevented or delayed by regular physical activity[24], maintaining a healthy diet[25], staying socially active[26], and participating in mentally stimulating activities[27]. Older adults can enhance physical, cognitive, and social functioning to reduce the risk of frailty[28].

2. **Recovery from frailty**: Dynamic resilience is essential for frail older adults with health challenges in their daily lives. Interventions promoting functional independence can improve overall well-being in frail older adults by helping them regain physical and cognitive function[23].

3. **Adaptation to changing circumstances**: Frailty can limit an older adult's ability to respond and adapt to new circumstances and stressful events[29]. Dynamic resilience can help overcome these limitations by utilizing available resources and support systems to promote adaptive strategies and emotional well-being.

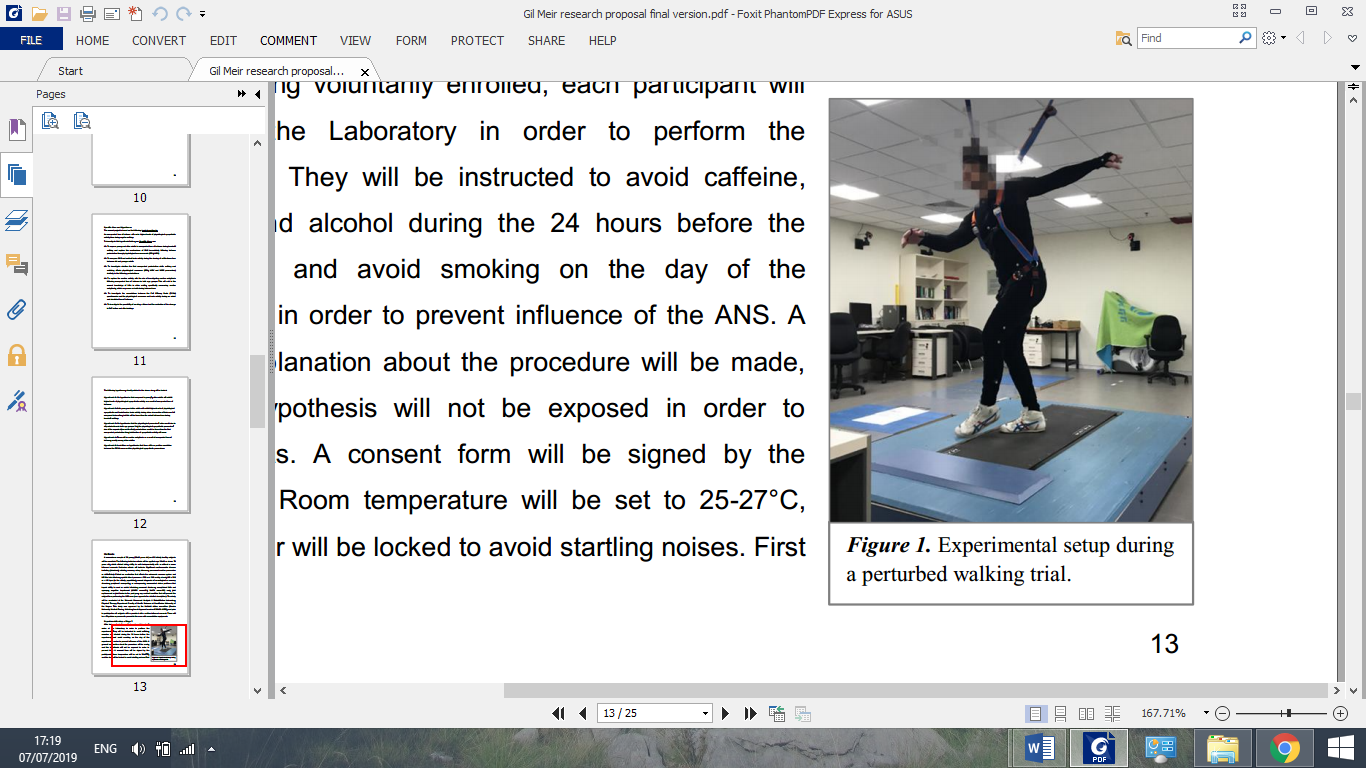
4. **Intervention**: Dynamic resilience is a crucial factor in intervention programs for older adults with frailty[30,31]. They can build resilience and regain functional independence to the best of their abilities through interventions tailored to improve physiological, biological, medical, nutritional, functional, and emotional well-being.

5. **Supportive Environments**: Creating supportive environments for frail older adults is crucial to foster their dynamic resilience[32]. The support includes accessible infrastructure, social support networks, healthcare services, and community programs that promote engagement, empowerment, and well-being.

**Proposal goal:** It is crucial to understand the mechanism underlying dynamic resilience to maintain and improve the health of our aging population. How can some older adults cope with physical and psychological stressors, whereas others cannot? Investigating dynamic resilience can mitigate the impact of frailty and enhance the overall well-being of older adults. Dynamic resilience is particularly relevant for older adults as they navigate the physical, cognitive, biological, emotional, and social changes accompanying aging. **We propose to understand the physiological mechanisms of dynamic resilience by identifying dynamic prognostic markers of resilience in older adults with the goal of predicting and detecting risk factors for frailty years before onset**.

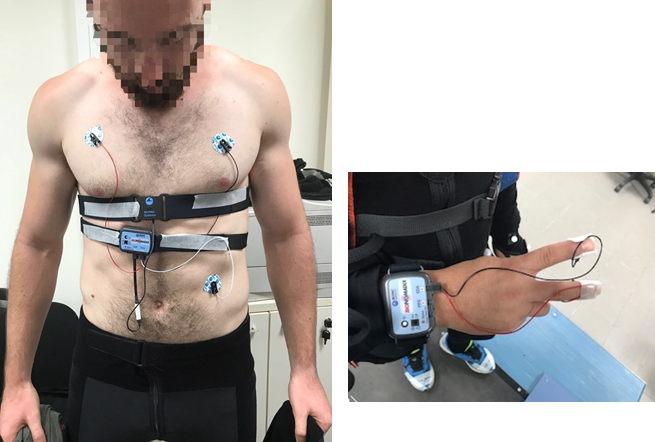
**Use of unexpected perturbations to assess autonomic nervous system activity:** Balance-threatening situations, especially unexpected balance perturbations, have a dramatic effect on autonomic nervous system activity (ANS), even in young adults. Previous studies measured autonomic nervous system activity under different conditions: perturbed sitting [33,34], simulated unperturbed standing using virtual reality[35], and real heights[36-38] in perturbed standing[36,37,39-41]. The studies included different tasks, such as walking on uneven surfaces and climbing stairs in populations at high risk of falls[42,43]. None of these well-designed studies investigated the relation to resilience among older adults.

**Preliminary Results**



***Figure 1:*** *Experimental setup. An example of balance recovery response during perturbed walking.*

During the past 20 years, PI Melzer's team developed novel examinations and testing protocols that measure balance function among older adults. Specifically, these examinations provide information about mechanisms of gait and balance function during the stress of an unexpected loss of balance event. We developed and built a novel motion platform, the Balance Measure & Perturbation System (BaMPer, patent PCT/IB2010/ 052079)[44], which provokes loss of balance while walking, simulating a real-world fall event. We can characterize responses following unexpected balance loss among older adults[45] and find that older adults reporting a recent fall cannot effectively recover from unexpected balance loss. In contrast, non-fallers show effective balance recovery[45-54]. We also find a significant increase in the total spectral power of lower-limb muscles during the first three seconds after perturbation [49], indicating fast-twitch muscle fiber use during recovery. In addition, we exposed 30 older adults to perturbations while standing. People with stroke demonstrated significantly lower fall and multiple-step thresholds, with 24 of 30 falling into the harness system during the experiment and most falls occurring toward the paretic side [50-52]. We further investigated responses to unexpected balance loss while concurrently performing a dual-task in older [53] and 13 younger adults [54]. In both groups, balance recovery parameters are similar in single-task and dual-task conditions, indicating that when the postural threat is substantial, such as unexpected balance loss during walking, recovery reactions are automatic and unaffected. Recently, we completed an MRI study [papers in preparation] demonstrating that associations between gray matter volume in prefrontal and sensorimotor ROIs with balance recovery stepping parameters change with age. The changes indicate the involvement of cognitive higher brain areas as well as the basal ganglia-cortical loop. Additionally, we find that reduced gray matter volume in the cerebellum is associated with a lower fall threshold in perturbed walking among older adults.



***Figure 2:*** *Demonstration of electrode placement. Left photo – the 3-lead ECG placement, with the respiratory strap. Right Photo– Electrodermal activity electrodes placed on the distal phalanx.*

In our recent study [paper in preparation], 34 young adults (27.2±2.3 years) were exposed to six unexpected balance losses during walking (**Figure 1**). We examined the skin conductance level (SCL) before and immediately after each perturbation by galvanic skin response or electrodermal activity (EDA) as a measure of the sympathetic nervous system (SNS) response. We also measured electrocardiogram (ECG) and heart and respiratory rates (**Figure 2**). We calculated the skin conductance response (SCR), defined as the change (delta) in the absolute value of the SCL before and after perturbations, which represents the phasic response of SNS. An SCR was determined as event-specific, a direct outcome of the stimulus if the increase started during a time window of 2-5 seconds after the perturbation. Predictably, we found that unexpected balance loss elicited a dramatic and significant rise in SNS response (**Figure 3A-C**). Interestingly, three different behaviors of ANS were observed: 1) **Steady level behavior** - This most common type of EDA behavior (58%) was characterized by a transient increase (phasic raise) of SNS activity immediately after each perturbation, which was followed by an immediate recovery to pre-perturbation level (**Figure 3A**); 2) **Ascending behavior** - Thirty percent of participants experienced increased EDA immediately after each perturbation, followed by an EDA decrease; however, EDA remained greater than the pre-perturbation level possibly indicating **less adaptive resilient behavior** (**Figure 3B**); and 3) **Descending behavior** - Twelve percent of participants experienced increased EDA immediately after each perturbation, followed by a reduced level compared to the pre-perturbation level indicating **highly adaptive resilient behavior** (**Figure 3C**)**.**

|  |  |
| --- | --- |
| A |  |
| B |  |
| C |  |
| ***Figure 3.*** *(A) The steady level wave-shaped graph, characterized by a steady SCL with transient peaks which are SCRs post–pre perturbations, (B) The ascending step-shape. These graphs present an ongoing increase in SCL following perturbations, (C) The descending step-shape where the SCL constantly decreases.* | |

Our results show that unexpected loss of balance while walking is a stressful event, even for young, healthy adults. Moreover, the results emphasize the need to conduct similar experiments with older adults. We predict unexpected balance loss will be a stressful event triggering SNS activity in older adults. ***These experiments will help us explore their adaptive/resilient behavior in a laboratory setting***.

Our Preliminary Results provide the basis for this proposal to investigate physiological, biological, medical, functional, and emotional markers of dynamic resilience among non-frail, pre-frail, and frail older adults. ***In this more extensive investigation of older adults, we propose to study adaptive/resilient behavior, which is the ability to cope with stressful laboratory-based events.*** To investigate dynamic resilience in older adults, we will recruit 60 older adults, with an equal number of frail, pre-frail, and non-frail. To the best of our knowledge, this is the first investigation to identify the physiological ANS response, biological (cortisol level), medical (The Charlson Comorbidity and Fried Frailty Indexes), physical (gait and balance), Nutritional (Mini Nutritional Assessment-Short scale) and emotional markers of dynamic resilience. ***The ISF program provides an opportunity to investigate and identify physiological markers of resilience in older adult populations to accurately predict dynamic resilience following a stress event and identify causal biological mechanisms underpinning the loss of human physiological resilience at multiple scales.*** This empirical work will be the basis for future intervention studies to improve resilience among older adults.

**Primary Hypothesis and Objective of the Proposal**

**We propose that older adults with low adaptive behavior to an acute event of stress, a laboratory-induced unexpected balance loss, will show significantly reduced dynamic resilience compared to older adults displaying high adaptive behavior*. Our overall objective is to identify physiological, biological, medical, functional, and emotional markers associated with adaptive behavior and dynamic resilience.***

**Specific Aims**:

We will achieve our objective through three specific aims.

**1. Behavior and resilience in non-frail adults.** We will assess whether non-frail older adults possess higher levels of adaptive behavior and dynamic resilience compared to frail and pre-frail older adults.

**2. Correlation between behavior and resilience.** We will examine whether a significant positive correlation exists between adaptive behavior, as measured by the laboratory markers, and dynamic resilience in older adults.

**3. Predicting dynamic resilience.** We will build a multivariate model estimating the contribution of each laboratory marker in predicting dynamic resilience and construct a weighted dynamic prediction score.

Identifying the relationships between acute and transient stress and persistent motor function would advance our knowledge of the biological and physiological mechanisms underlying dynamic resilience. It would allow us to identify physical resilience mechanisms.

**Methods**

We will recruit sixty older adults (20 frail, 20 pre-frail, and 20 non-frail, n=60) from the community and among in-patient and out-patients at the Geriatric Department at Soroka Hospital in Beer-Sheva. All older adults will undergo a Mini Mental State Examination and medical history interview for exclusion. Our exclusionary criteria are being 65 years old and older, CNS trauma, including loss of consciousness for greater than 30 min, seizures, and degenerative disease or severe medical conditions, including stroke, Parkinson's disease, ALS, and other neurogenetic diseases. We will exclude subjects with an active malignancy within 24 months prior to examination or metastatic cancer, severe cognitive impairment (Mini Mental State Examination <20), or any medical reason such as a known or suspected inability to comply with the protocol. After signing a written informed consent, obtained according to the Declaration of Helsinki and the ethics committees, we will administer the following examination for research purposes only.

**Experimental Setup**

**Stage 1 – Preparation protocol and quiet sitting**

We will set the laboratory room temperature at a comfortable level according to the participant's preference and close the door to avoid startling noises. The skin of the participants' 2nd and 3rd distal phalanx of the right hand will be cleaned using cotton and alcohol. Dermal electrodes (detailed later) will then be placed for EDA recording. Using the Biopac system (Biopac Systems Inc. Goleta, CA, USA) for the ECG recording, we will place three electrodes with a 3-lead (I, II, III) configuration on both sides of the upper chest and the left side of the lower abdomen. The respiratory strap will be placed on the xiphoid process line slightly tightly, allowing the participant to breathe normally. We will affix all loose cables to avoid mechanical noise. Participants will rest in a comfortable seated position for five minutes for baseline recording. The room will be quiet with dim light in the absence of another person.

**Stage 2 – Perturbed standing and walking tests**

We will conduct a total of six unexpected perturbation trials for each participant using two task conditions: 1) six perturbed standing trials and 2) six perturbed walking trials. The participants will be strapped into a harness hung from the ceiling and designed to allow free joint motion during the trials. The harness permits balance loss trials but prevents ground contact and injury in a fall. We will instruct the participants to stand (1st task condition) or walk (2nd task condition) on the BaMPer system. No handrails are mounted on the perturbation system, so arms and hands movements are unconstrained (**Figure 1**). Each condition includes 12 unexpected surface translations as perturbations, six during the standing protocol and six during the walking protocol. During the standing trials, the participants will stand in a narrow base stance, with their feet placed together. During the walking trials, a habitual self-selected comfortable walking speed will be chosen for participants while wearing their own comfortable shoes. We will then perturb both task conditions with 30 to 45-second intervals between tasks. A research student familiar with system activation will sit at a computer in front of the BaMPer system and manually mark the time of the perturbations online for future analysis. Another student will stand near the participant to prevent a fall or injury. For both task conditions, we will instruct participants "to try to avoid a fall in case you lose your balance". During the walking protocol, we will videotape the experiment using a Hero4 GoPro camera.

**Proposed outcome parameters:**

1. **ANS response to stress after balance loss**

We will collect physiological measurements of EDA, ECG, and respiratory rate using the MP160 Data Acquisition System (DAQ). Two Bionomadix wireless transmitter + receiver pairs will be used for 1) ECG + Respiratory and 2) EDA. In addition to the Bionomadix respiration transducer, we will use EL507 electrodes (0.5% chloride salt) for EA measurements and EL503 pre-gelled electrodes for ECG (Biopac Systems Inc. Goleta, CA, USA) (**Figure 2**). Sixty seconds before the first perturbation and after the second perturbation, the subjects will walk without external perturbations. This protocol will allow us to examine the effect of unperturbed standing and unperturbed walking on the physiological parameters. The participants will rest for five minutes after completing the protocol on the BaMPer system.

We will collect physiological measurements of EDA, ECG, and respiratory rate 60-seconds before the first perturbation and after each of six perturbations using the MP160 DAQ, allowing us to examine the three behaviors: 1) steady level behavior, 2) descending behavior, and 3) ascending behavior. We will collect data during all experiment stages using the Biopac system. We will then extract from the raw data graphs of physiological parameters over time as an EDA graph of absolute values of microSiemens (μS) and ECG as a standard strip (mV).

### **EDA parameters:**

1. We will measure SCL by the tonic component of the skin conductance, which represents the basic autonomic arousal level.
2. We will determine SCR, which is the delta of the absolute value of the SCL following the perturbation. An SCR will be determined as an event-specific, direct outcome of the stimulus if the rise starts 2-5 seconds following the perturbation event[55].
3. We will measure the SCL value at the beginning and the end of the rise (μS) (as shown in **Figure 3A-C**).
4. We will determine the relative rise of SCL calculated as the ratio percent SCR/SCL. Based on our previous experiments, we will use log-transformation of the data to reach normal distribution. The physiological meaning of the ratio is the magnitude of the stress response compared to the basal stress level prior to the stimulus. We consider this parameter particularly valuable because it "normalizes" significant between-subject differences in EDA levels.

**ECG activity**

#### **Heart Rate values:**

1. We will calculate heart rate (HR) delta post-perturbation as the absolute delta (beats/min) between the single lowest value in the three seconds preceding perturbation (pre-perturbation HR) and the highest single value (HR Peak) within 2-20 seconds from the time of perturbation.
2. The time to peak is the time (sec) between perturbation and the following HR peak.
3. The HR delta of means is the change between the mean HR 10 seconds prior to the perturbation (the last window pre-perturbation) and the greatest mean value of the 10-second window 5-30 seconds post-perturbation.
4. Time to recovery is the time (s) between the peak HR and when the HR decreased to the pre-perturbation HR (or 0.5 BPM above).

#### **Heart Rate Variability values**

We will calculate heart rate variability (HRV) parameters using the 10-second sliding windows, the minimal time range for some widely used HVR parameters. All calculations will use the normal and interpolated beats.

1. **The root mean square of standard deviation (RMSSD).** This parameter is often considered reliable for short and ultra-short measurements[56] and is highly associated with the parasympathetic tone[57,58].
2. **The standard deviation of normal-to-normal beat intervals (SDNN).** This parameter is reliable under specific conditions for short-term measurements and is linked to sympathetic and parasympathetic divisions[58,59].
3. **Stress biomarkers**

We will test whether unexpected loss of balance affects stress system activity, namely the hypothalamus-pituitary-adrenal (HPA) axis and low-grade inflammation. We will examine the concentration of the stress biomarkers cortisol and alpha-amylase, the inflammatory markers C-reactive protein and interleukin (IL)-6, and tumor necrosis factor TNF-alpha before and after an unexpected balance loss in the laboratory. Stress system activitywill be assessed by the activity of the HPA axis using non-invasive saliva samples that measure salivary cortisol[60]. We will determine IL-6 and TNF-a levels using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA).

1. **Proxy biomechanical measures of fall risk**

A number of measures have been proposed to evaluate reactive balance control for effective balance response[61]. We will use 3D motion analysis (Vicon system Ltd.) to determine the center of mass relative to the base of support during and immediately following perturbations. In addition, we will measure single-step and multiple-step fall thresholds, which we find identify older adults who recently reported a fall[45-54].

1. **Gait speed**

Gait speed is the best predictor of survival among older adults[62]. We will measure gait speed with a digital stopwatch using a 10-meter walking path with a 5-meter measurement section bordered on each side by 3-meter preparation sections. At the start of measurement, we will verbally instruct subjects to "Please walk normally", and their walking speed will be measured (m/s) using the recorded time (s).

1. **Brain markers**

Our recent study [papers in preparation] shows that aging is associated with structural changes in many brain regions[63]. We will use advanced neuroimaging techniques to explore the structural differences between individuals with high and low dynamic resilience and examine how dynamic resilience corresponds to age-related changes, namely brain shrinking. We propose to use voxel-based morphometry (VBM) to investigate regional and ROI brain volume differences among the groups. We also propose a fMRI investigation, which will use motor imagery and action observation to elicit brain activity related to reactive balance control. We will then project the video clip recorded during the walking protocol during the fMRI imaging.

**Questionnaires to be utilized:**

**The Charlson Comorbidity Index (CCI)**. The CCI is a simple and widely used index for assessing comorbidities. It is administered in 5-10 minutes and is most commonly studied for predicting mortality [64]. The CCI scale [65] will be used to calculate the comorbidities of subjects at enrollment.

**Mini Nutritional Assessment-Short Form (MNA-SF).** The MNA-SF[66] will evaluate the nutritional status of the subjects with three classifications: 0–7 points are malnourished, 8–11 points are at risk of malnutrition, and 12–14 points are well-nourished[67]. Participants will be divided according to MNA-SF score into well-nourished and malnourished/at risk of malnutrition.

**The MiC questionnaire for dynamic resilience.** The MiC questionnaire[68] contains 34 items addressing a variety of factors influencing resilience in older adults(QMU and NHS Lothian[68]24). The items are split across two distinct subscales, one assessing the individual determinants of resilience (IDoR) and one assessing the EDoR. The items address participant perceptions of self-care, leisure, work, responsibilities, social environment, resources, habits, values, self-efficacy, motor skills, communication skills, and process skills. The questionnaire grade will serve as our gold-standard measurement for resilience.

**Fried frailty phenotype.** The Fried frailty phenotype[69] is the most popular measurement of frailty. The index considers frailty by its physical characteristics, or "phenotype". The index defines frailty as the presence of shrinking (self-reported unintentional weight loss of 4.5kg or more in the last year), weakness (low grip strength), exhaustion (self-reported), slowness (slow walking speed), and low physical activity. Older people without the five indicators are characterized as robust and non-frail. Those with one or two indicators are hypothesized to comprise intermediate or pre-frail people, whereas those with three or more indicators are considered frail[70].

**Dundee Stress State Questionnaire (DSSQ).** The DSSQ[71] is a self-reported measure used to assess an individual's stress level. It consists of 15 items that capture different aspects of stress, including perceived stress, physical symptoms, and negative emotions[71]. Respondents rate each item on a four-point scale indicating the extent to which they experience stress-related feelings or symptoms.

**Statistical analysis and sample size estimation**

Our statistical analyses will be conducted in three phases.

**First,** we will investigate the association between a stressful environment and resilience/adaptive behavior by using a two-way analysis of variance with interactions for each laboratory parameter (ANS, Stress biomarkers, biomechanical measures, gait speed, and brain markers) and overall questionnaire grades (MiC, Fried frailty phenotype, DSSQ, and MNA) as a dependent variable. In contrast, the area of living and age group will be independent variables. Non-normally distributed dependent variables will be transformed to achieve normality. P-values will be adjusted for multiplicity using the Benjamini and Hochberg false discovery rate (FDR) controlling procedure[72].

**Second,** we will assess the association between laboratory adaptive behavior measurements and dynamic resilience using the overall MiC questionnaire grade. We will initially do univariate analysis using the Spearman correlation coefficient and test with FDR adjustment for multiplicity. Next, we will use a multivariate generalized linear model with resilience as a dependent variable and adaptive behavior measurements, area of living, and age as independent variables to identify resilience-associated markers. The link function for the generalized model will be selected in accordance with the distribution of the resilience variable.

**Third,** we will use the multivariate model to construct the dynamic dRePS.The score will be defined as a weighted summary of the biomarkers identified by the model, which are ANS and cortisol responses, balance and gait speed, and brain markers. We will determine weights by the model coefficients. We will then use statistical model selection methods such as forward selection, backward elimination, least absolute shrinkage, and selection operator (lasso)[73,74] to refine the prediction model. The model will be validated on an independent group of participants.

**Sample size**. We will include a total of 60 participants to achieve a statistical power of 0.93 at a significance level of 0.05, based on two-way ANOVA utilizing six combinations, represented by three frailty groups by two task conditions (standing and walking). The dRePSwill be validated on an independent group of 30 participants not included in the model development phase.

**Possible limitations**

The CCI's role in predicting long-term clinical outcomes in elderly patients is controversial[12–15]. Criticisms include the lack of consideration of disease severity, functional impairment associated with different diseases, and the omission of nutritional and social assessments[16]. Similar to comorbidities, nutritional status is confirmed by numerous studies to have a strong association with long-term mortality in the elderly, and it is believed that good nutritional status is significantly correlated with better prognosis[17–19]. Poor nutritional status weakens the body's immune system and increases the susceptibility to infection-related diseases. While comorbidity and nutritional status are distinct conditions, they are closely related [20–22]. Therefore, combining comorbidity assessment with nutritional evaluation may be more effective for predicting mortality.

**Budget timeline by activities**

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Proposed timeline for the study.** | | | | | | | | | | | | | | |
|  | **Activity** | **Subj.** | **Year I** | | | | **Year II** | | | | **Year III** | | | |
| 1 | Preparation for Helsinki submission | *n/a* |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | Helsinki submission and insurance | *n/a* |  |  |  |  |  |  |  |  |  |  |  |  |
| 3 | Recruit subjects | *60* |  |  |  |  |  |  |  |  |  |  |  |  |
| 4 | Screen & test subjects | *60* |  |  |  |  |  |  |  |  |  |  |  |  |
| 5 | Database entry | *n/a* |  |  |  |  |  |  |  |  |  |  |  |  |
| 6 | Analysis of data | *n/a* |  |  |  |  |  |  |  |  |  |  |  |  |
| 7 | Development of the dRePS | *n/a* |  |  |  |  |  |  |  |  |  |  |  |  |
| 9 | Reports and paper writing | *n/a* |  |  |  |  |  |  |  |  |  |  |  |  |
| 10 | Study finalized | *n/a* |  |  |  |  |  |  |  |  |  |  |  |  |

**Funding and Activities Summary**

***Total cost and duration of the proposed project.***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Salaries** | **USD** | **Year I** | **Year II** | **Year III** | **Total ($)** |
|  |  |  |  |  |  |
| *PI (30%)* | 3 years |  |  |  |  |
| *PhD students (100%)* | 3 years | 21,000 | 21,000 | 21,000 | **63,000** |
| *1 MS student (100%)* | 3-years | 16,344 | 16,344 | 16,344 | **49,032** |
| *Lab engineer (100%)* |  | 10,000 | 10,000 | 10,000 | **30,000** |
| ***Total salaries*** |  | ***47,344*** | ***47,344*** | ***47,344*** | **142,032** |
| *MRI hours* | 60 subjects x 500$ | 15,000 | 15,000 | - | **30,000** |
| *Subject payment* | 60 subjects x 40$ | 1,200 | 1,200 | - | **2,400** |
| *Helsinki submission* | 1 | 1,500 | - | - | **1,500** |
| *Study insurance costs* | 1 | 5,000 | - | - | **5,000** |
| *Cortisol kits* |  | 5,000 | 5,000 |  | **10,000** |
| *Physiological system* | 1 | 20,000 | - | - | **20,000** |
| *Supplies & materials* |  | 20,000 | 20,000 | 20,000 | **60,000** |
| *Other direct cost* |  | 5,000 | 5,000 | 5,000 | **15,000** |
| ***Total other direct*** |  | ***66,200*** | ***46,200*** | ***25,000*** | **138,500** |
| ***Total direct*** |  |  |  |  |  |
| *Indirect* |  |  |  |  |  |
| **Total** |  |  |  |  |  |