1. Project Title (150 Character Limit including spaces and punctuation):

Decelerating the aging process through targeted environmental intervention among a multi-ethnic elderly population in Israel: an epigenetic approach

2. Executive Summary (1,300 Character Limit including spaces and punctuation): \*

Aging refers to progressive corporeal and functional deterioration at the molecular, cellular, and systemic levels. By evaluating a cohort of exceptionally long-lived individuals (ELLIs), we have identified distinctive patterns of DNA methylation associated with protection against aging-related disease, thus extending the healthy human lifespan. Importantly, these patterns are epigenetic in nature, meaning that they are not hard-wired into the genome and can instead be shaped by environmental exposures. This project thus has the following aims: 1) to determine whether specific environmental manipulations can decelerate the aging process; 2) to establish whether DNA methylation can serve as a biomarker that reflects the benefits of such manipulation; and 3) to explore the heritability of these changes and the mechanisms whereby they function.

To address these aims, we will employ hyperbaric oxygen therapy (HBOT), which is already well-established as a treatment for decompression sickness, to create an artificial space capable of decelerating the aging process, as occurs naturally among ELLIs. We will then compare the epigenetic profiles (epigenomes) of elderly individuals exposed to HBOT to those of ELLIs in order to tease out the regulatory mechanisms that protect ELLIs against these deleterious aging-related processes. We will also analyze model mice exposed to HBOT to gain a detailed understanding of these protective mechanisms.

This project will identify new circulating biomarkers of aging deceleration, thus directly supporting the design of therapeutic approaches to slowing the aging process and associated diseases. Achieving these goals will offer invaluable new insight into the mechanisms of aging and the interactions between the human body and the environment, helping us to close the gap between life expectancy and healthy life expectancy.

3. Project Description (4,000 Character Limit including spaces and punctuation): \*

Please describe the work/activities you will undertake in your project.

4000 characters remaining

Our main aim is to define novel circulating biomarkers of decelerated aging, to explore how they link lifestyle to longevity, and to determine whether they are heritable and can promote trans-generational healthy aging.

We will use state-of-the-art Infinium MethylationEPIC beadchip and EPIC array tools with HBOT to gain insight into epigenetic changes detectable in DNA samples collected from CD34+/Lin- cells in whole blood samples. We will tie these epigenomic profiles to data derived from comprehensive cognitive assessments (NeuroTrax MindstreamsTM, NeroTrax Corp, NY), advanced brain imaging analyses (functional MRI+DTI+perfusion), and physiological cardiopulmonary exercise testing (CPET). To gain a robust understanding of the mechanisms that trigger the decelerated aging, we will expose old and young mice to HBOT. Our proposed aims are:

**Aim 1: To establish the effect of HBOT on the human epigenome.**

**1a.** EPIC arrays will be used to cross-sectionally monitor epigenomic changes in elderly individuals undergoing HBOT (baseline, during treatment, post-treatment), and epigenome-wide association studies will be conducted to detect candidate epigenetic sites (epiloci) correlated with the results of cognitive tests, functional MRI, and CPET. Differentially methylated loci will be validated with the Sequenom MassArray technology.

**1b.** To further explore the relationship between aging and HBOT, we will incorporate a younger cohort (45-55 years old) that will be treated similarly to the elderly group. Comparisons between the three groups (ELLIs, elderly, and young) will be conducted to detect aging-related effects.

**Aim 2: To identify molecular networks and longitudinal trajectories predictive of decelerated aging following HBOT.**

We will Integrate the high-dimensional data from Aim 1 to define molecular networks and trajectories associated with recovery after HBOT. Integrative approaches will include model-based statistical analyses to score genes for their association with healthy performance based on the strength of genetic associations, related phenotypes, and, crucially, the genetic interactions that account for the complexity of healthy aging.

**Goal 3: To establish candidate epiloci that can be prioritized for further investigation.**

**3a.** The intersection of two fields of research (recovery therapy and ELLIs as a model of successful aging) will further our understanding of the complex aspects of a healthy lifespan by identifying loci that, when epigenetically altered, may have important ramifications for the transition between aging (elderly before HBOT) and a healthy lifespan (similarly methylated loci in both the post-HBOT recovery and ELLI cohorts).

**3b.** Based on epigenetic results from our ELLI cohort, we will compare the results from treated and untreated subjects from Aim 1A to these ELLIs to detect epiloci that may affect recovery or maintenance of healthy aging in both of these groups.

**3c**. We will test the heritability of candidate epiloci by analyzing the offspring of ELLIs.

**Aim 4: To clarify the epigenetic mechanisms underlying healthy aging in mice.**

We will conduct an EPIC array analysis of mice at 4 and 12 months after HBOT treatment, and will assess methylation profiles in these animals (baseline, during treatment, post-treatment, 1-month post-treatment, and lifelong follow-up) to understand the mechanisms governing treatment-related epigenetic effects. We will sacrifice 20 animals at each time point and evaluate them at the physiological, biological, and molecular levels. We will seek to detect associations between these findings and epigenomic profiling results to more fully understand the mechanistic basis for aging and the slowing of the aging process.

**By comparing studies of ELLIs, elderly populations undergoing HBOT, and animals exposed to HBOT, we will comprehensively identify markers of decelerated aging that can be leveraged to monitor and improve healthy aging.**

4. Statement of Significance (1,300 Character Limit including spaces and punctuation): \*

Describe the current conditions in the field(s) relevant to the project, identify the problems that the project will address, and articulate the specific opportunity that your project presents.

1300 characters remaining

While prior work has highlighted the impact of HBOT on human physiology and neurological function at the genetic level and the link between environmental exposures and genetic predispositions, the epigenetic determinants of HBOT treatment outcomes have yet to be fully clarified or tied to these key biological outcomes. This study will be among the first to establish the mutual dependence of epigenetic changes and decelerated aging in humans and animal models, exploring the heritability of these changes while providing direct insight into how specific epiloci are related to cognitive function and physiological performance. By uniting all of these facets of the epigenetics of aging under a single project, we will provide a holistic framework for psychophysiological investigation and treatment using a combination of novel technologies that are ideally suited to use when conducting epigenetic analyses, cognitive and physiological assessments, and HBOT treatment. By leveraging these technologies, a robust cohort of patients (ELLIs and 48 - 85-year-olds), and animal models, we are ideally positioned to discover the role of epigenetic changes across the lifespan on elderly individuals and their cognitive function, enabling us to decipher the effects of the environment on healthy aging.

5. Outputs (1,300 Character Limit including spaces and punctuation): \*

Outputs (sometimes called "deliverables") are important events and work products that your Project activities (described in #3, above) will lead to, and which are necessary in order for you to make progress towards your proposed Outcomes (#6 below). Please provide a list of the outputs you intend to produce.

1300 characters remaining

Our efforts towards completing the four core Aims of our project will yield a large volume of data that will be thoroughly analyzed to guide the project and to accomplish the outcomes discussed in the following section. The key outputs from this project will include:

1. Methylation data pertaining to 870k epigenetic loci in 60 subjects across multiple stages of the treatment process (before, during, and after HBOT treatment).
2. A collection of multiple physiological and anthropometric determinants for each participant.
3. A collection of multiple brain imaging and functional results for each participant collected before, during, and after HBOT treatment using MRI+perfusion+DTIA and CPET analyses.
4. Methylation data pertaining to approximately 870k epigenetic loci in 100 HBOT-treated mice of different ages.
5. A collection of multiple mouse tissues harvested across the HBOT treatment period for mice in each age group.

6. Outcomes (1,300 Character Limit including spaces and punctuation): \*

Outcomes (sometimes called goals, results, or impacts) are the specific and identifiable changes that you expect your Outputs will bring about (or contribute to bringing about) within 5 years of your project's end date. These should describe what the success of your project would look like. Please provide a list of the outcomes you expect to come about as a result of your outputs.

1300 characters remaining

Based on our preliminary results, prior knowledge, and thorough searches of the literature, we expect that our proposed project will yield the following outcomes:

1. We will identify candidate epiloci that serve as reliable biomarkers of HBOT-mediated aging deceleration as evidenced by improved cognition, reduced frailty, and better age-associated disease maintenance and recovery.
2. Will will define molecular networks that act, both together and in parallel, to delay aging-related deterioration, laying the groundwork for the improved understanding of the multifaceted complexity of the aging process.
3. By prioritizing specific epiloci and studying model mice, we will gain comprehensive insight into the fundamental epigenetic responses to arise following HBOT treatment and their heritability. By clarifying the mechanisms mediating these responses, we will be able to design similarly potent environmental interventions that can slow the aging process and prolong the healthy lifespan.

The biomarkers that we identify will be amenable to easy assessment in primary care settings, advancing society ever closer to an understanding of how aging occurs and how we can slow this process to prolong healthy life expectancy and to facilitate aging with grace.

7. Capacity for Success (1,300 Character Limit including spaces and punctuation): \*

Explain why your team and/or organization is positioned to be successful in this project.

1300 characters remaining

Our organization is ideally positioned to successfully complete this project for several key reasons:

1. Close interactions among PIs with complementary fields of expertise, including epigenetic technologies and analysis (Prof. Atzmon), the enrollment of elderly study subjects (Prof. Dwolatzky), all aspects of HBOT (Prof. Efrati), mouse studies using HBOT (Prof. Asheri), and computational and biostatistical analyses (Dr. Judith Somekh).
2. Research infrastructure is already in place due to ongoing collaborations among the PIs through multiple University of Haifa, Rambam Health Care, Assaf-Harofeh Medical Center, and Tel Aviv University programs.
3. Access to state-of-the-art high-throughput epigenetics, HBOT, and bioinformatics analyses as well as brain function analytical techniques including computerized neurocognitive testing and functional MRI+perfusion+DTI and CPET.
4. Animal studies will enable us to clarify the mechanistic determinants of HBOT treatment outcomes.
5. We have access to an ideal model population of ELLIs and their offspring, allowing us to study slow, healthy aging.

This project will spur many future studies that will further define the epigenetic regulation of healthy aging, potentially guiding the selection of promising therapeutic approaches.

8. Relation to Sir John Templeton's Donor Intent (1,000 Character Limit including spaces and punctuation): \*

To learn more about the Foundation's Funding Areas please visit our Funding Areas page.

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We aim to promote healthy aging by studying HBOT, which is a remarkable treatment that has already been shown to slow the aging process. Most studies of HBOT to date have focused on human physiology. We instead propose to explore the fundamental epigenetic mechanisms that regulate this phenomenon and the heritability of these epigenetic changes. We believe that our aims align perfectly with Sir John Templeton's Donor Intent for several reasons. For one, using HBOT as an environmental exposure will demonstrably affect the aging process, cognitive function, and aging-related disease incidence. Importantly, by harnessing several new technologies, we will discover epigenetic biomarkers to guide the development of diagnostic platforms for monitoring aging and deterioration, thus improving our ability to prolong the healthy human lifespan. Overall, this will open new avenues to the inter-generational protection of the well-being of individuals, their offspring, and society as a whole.