

Anti-Aging Behavioral and Lifestyle Modification Strategies Produce Positive Epigenetic Changes in Older Adults

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ABSTRACT

Purpose: Many studies demonstrate the efficacy of individual or concurrent complementary and alternative medicine strategies, as well as lifestyle changes, to improve physical health and psychological well-being. A four-month long observational study, enrolling 18 adults over 60 years of age, was conducted to evaluate a multifaceted lifestyle modification regimen for improving older adults' quality of life and to slow biological aging.

Materials and Methods: The study was based on a customized regimen of physical strength exercises, a plant-based diet, intermittent fasting, stress reduction and education to deconstruct negative cultural views about biological aging. Education and self-motivation tools were introduced regularly to encourage positive attributes of personal self-fulfillment. Aging markers including physical endurance metrics, cardiovascular and lipid chemistries and Grim Age DNA methylation tests were evaluated.

Results: All of the study's 18 participants were in a good state of health, free of any diagnosed serious illnesses and were not taking any medications. As expected, there were no significant change ($p < 0.05$) in the cardiovascular blood markers except total cholesterol ($p = 0.0125$) and LDL ($p = 0.025$). DNA methylation Grim Age tests for the DNAmFitAge suggest an estimated 5.9-year biological age reduction calculated to chronological age with a 1.3-year standard error ($p = 0.0003$). Hand-grip strength (DNAmGripAge) increased (0.58 years) when calculated without factoring in chronological age ($p = 0.00001$). There were only small signs of positive methylation reversal in the DNAmAge (-1.582), DNAmGrim-Real (-1.603), DNAmGrin-Predicted (-1.731), DNAmGrim2-Real (-1.441) and DNAmGrim2-Predicted (-1.589), but no significant trend across the group ($p > 0.05$). There were mean endurance and stamina improvements in muscle gain and bone density.

Conclusion: Results demonstrated that a robust lifestyle modification program, with an emphasis on education and positive psychosocial reinforcement, provides an effective strategy as an anti-aging and wellness model to increase physical, mental and emotional health in older men and women

Keywords: Aging, DNA Methylation, Lifestyle Modification, Diet, Exercise, Education

1. Introduction

In recent years, there has been growing interest in “lifestyle medicine^{1,2}.” Lifestyle, especially pertaining to physical activity and diet, has been assessed to have a positive association on the physical and mental health of elderly citizens. Physical exercise is highly significant for improving several cognitive functions for people over 65 years of age³. At the 2013 International Conference on Nutrition and the Brain, speakers indicated a major shift towards advocating diet and exercise as an evidence-based strategy for preventing illnesses associated with cognitive decline⁴. Switching to a plant-based diet, especially in geriatric medicine, is also reaching a larger consensus⁵. Research into the role of dietary habits and lifestyle behaviors, particularly in comparing omnivorous and vegetarian diets, upon biological aging markers, such as DNA methylation, are revealing promising associations to develop effective drug-free interventions⁶.

Despite the many studies conducted that underscore the effectiveness of individual or concurrent complementary and alternative medicine (CAM) strategies, coupled with lifestyle changes, to enhance overall health, there is a distinct gap in research evaluating the collective impact of a comprehensive approach that integrates simultaneous CAM interventions and lifestyle behavioral modifications to slow the progression of aging, to ameliorate disease and to bolster overall well-being.

Aside from biological aging, one of the present study’s hypotheses is that aging is also a socially acquired mindset with cognitive factors related to perceptions about self and others⁷. Stereotype Embodiment Theory (SET) is a theoretical framework that focuses on how cultural stereotypes about aging can influence the thoughts, feelings and behaviors of individuals as they age. This theoretical model, developed by Yale University epidemiologist and psychologist Dr. Becca Levy, explains how processes within cultures holding to strong negative beliefs about aging are internalized over people’s lifespan and shape their self-perceptions that in turn influence their physical and cognitive functioning. A society that reinforces adverse perceptions and beliefs about declining age, frailty, cognitive decline and dependency during senior years can accelerate adverse aging symptoms such as memory decline and biological longevity^{8,9}. In addition, negative stereotypes reinforce a sense of perceived control, a loss of independency and pessimism over a variety of behavioral factors in one’s later years¹⁰. SET proposes that the internalization of such negative stereotypes have real, measurable effects on physical and mental health. It also explains health-related behaviors; older adults who embody negative stereotypes are shown to be less likely to engage in health-promoting habits and more prone to isolation and loneliness. Aging can therefore reflect a self-fulfilling prophecy based upon the way older adults evaluate aging stereotypes. These negative social stereotypes become social constructs that are internalized and operate from the unconscious¹¹.

SET suggests that interventions that challenge negative stereotypes and advance positive images of aging can develop more beneficial self-perceptions. If an individual can reverse the negative stereotypes being held, then physical and psychological health and quality of life can be preserved and be enjoyed by people at any age. A comprehensive holistic lifestyle modification program, with an emphasis on education, counseling and alternative methods to instill greater stress-free well-being could result not only in improved health outcomes and the slowing or

reversal of diseases, but also in measurable epigenetic changes indicating the potential for longer life expectancy.

Introducing a comprehensive education curriculum to challenge pessimistic aging stereotypes and promote positive perceptions forms the cornerstone of the study’s comprehensive regimen, imparting a deeper understanding of the intricate interplay between personal habits, adverse beliefs about aging and their health outcomes. By providing individuals with the practical knowledge to make informed lifestyle choices, the regimen empowers trial participants to take personal responsibility for their health. Furthermore, an emphasis on motivation is paramount as is employing proven strategies such as goal-setting, recognizing milestones and visualizing the enduring benefits of positive, constructive habits. Research shows that adopting several health behaviors, notably physical exercise, a healthy diet and refraining from smoking and alcohol, can delay all-cause mortality by 11-14 years¹². This motivational aspect follows along the lines of the benefits of positive thinking training, which has been shown to improve personal resilience and life satisfaction in older adults¹³. The goal is not only to initiate a fundamental change in personal outlook but to sustain enthusiasm throughout the transformative journey to ameliorate and/or reverse adverse health conditions due to behavioral choices and habits, such as an unhealthy diet, lack of exercise and sedentary lifestyle, poor decision making, an inability to cope with stress, etc.

Cultivating a positive outlook on life is also an integral component for a comprehensive holistic approach. Encouraging individuals to focus on the optimistic aspects of their health and instilling a growth mindset contribute to building psychological resilience.

There is evidence that optimistic people generally have a higher quality of life and health compared to those who are pessimistic; optimistic individuals are more easily able to adapt themselves to health-promoting habits¹⁴. Psychosocial optimism has also been associated with an 11-15 percent longer lifespan and greater chances of reaching “exceptional longevity” of 85 years and beyond¹⁵. For older adults, this shift in perspective, from perceiving lifestyle changes as restrictions to viewing them as opportunities to increase optimism towards an individual’s personal growth and well-being, establishes a solid foundation for long-term success and has been shown to reduce the incidence of chronic illnesses over time¹⁶.

Moreover, a comprehensive lifestyle modification regimen extends beyond theoretical concepts to incorporate practical strategies. In the present trial this included introducing an evidence-based nutrition plan, an organic, gluten-free, plant-based diet, fruit and vegetable juicing, nutritional supplementation, regular physical activity tailored to individual needs, reinforcing personal discipline through intermittent fasting, stress management through mindfulness, meditation and mind-body practices (yoga and Tai chi) and strategies for ensuring sufficient sleep. Health education about the fundamental principles of biological aging and the self-examination of personal beliefs and attitudes was designed and based upon existing medical literature.

The present study specifically looked at blood biomarkers associated with aging associated with cardiovascular health and glycemic management. The DNAmGrimAge epigenetic clock

was used to measure participants' DNA methylation (DNAm) changes before and after an intensive physical and behavioral modification regimen. Research has shown that epigenetic clocks are sensitive to lifestyle factors¹⁷. The DNAmGrimAge clock has been shown to most accurately measure epigenetic age acceleration based on DNAm-based estimators of plasma proteins as predictors of lifespan¹⁸. This preset study also included the new biological age indicator DNAmFitAge developed by the Epigenetic Clock Development Foundation at the University of California at Los Angeles. DNAmFitAge measures fitness parameters including maximal oxygen intake (DNAmVO2max), forced expiratory volume as an index of lung function (DNAmFEV1), walking speed (DNAmGait) and hand grip strength (DNAmGrip)¹⁹. This new DNA longitudinal test can better distinguish degrees of fitness than earlier DNAm biomarkers²⁰. It is the only epigenetic clock to utilize mobility, strength, respiratory capacity and physical endurance as a correlate to DNAm with mortality risk.

The purpose of this observational trial was to determine whether a comprehensive lifestyle modification regimen goes beyond the potential limitations of narrow isolated approaches for biological aging. Unlike other natural health protocols investigated to offset adverse effects of cellular aging, the current study incorporates and emphasizes a robust educational component to increase trial participants' personal motivation, a positive outlook on their lives and personalized incentives. Past studies conducted by the authors have shown that personal initiative and an optimistic psychology has been crucial for achieving noticeable quantitative results. Without personal motivation and incentive sustained over a period of time, older adults are more than likely to fall back into prior negative behavioral patterns that again reinforce rapid biological aging. This particular protocol has the potential to equip individuals with the tools to navigate a unique and transformative path toward achieving better health outcomes and well-being that have direct impact upon their biological aging.

2. Methods

Starting in 2019, the authors conducted six successive discrete small observational clinical trials, averaging twenty individuals, who were enrolled to participate in anti-aging health retreats at an idyllic rural retreat center in Mineola, Texas. For the sixth study, all volunteers were interviewed and questioned by one of the study's authors to evaluate their state of health, pre-existing illnesses and whether or not they were willing and motivated to commit to a comprehensive lifestyle modification program as part of a 4-month clinical study. Eighteen individuals were eligible to enroll based upon the screening criteria. Participants' ages ranged between 61 and 80 years of age, with an average age of 68.8 years. Each participant was in a good state of health, lived a relatively healthy life, was free of serious comorbidities and pre-existing conditions.

During the first two weeks, the participants followed an intensive in-residence daily regimen at the retreat center; during the remaining 14 weeks, participants individually completed the trial's regimen at their home of residence with twice weekly remote educational and counseling support forums led by two of the study leaders.

All participants were required to have a negative PCR test for the SARS-CoV-2 virus prior to entering the campus.

An enrollment criterion was that no participant was taking a prescription medication. On campus, participants were instructed and guided to follow an identical and disciplined lifestyle and behavior modification program. The selected regimen's natural therapies were based on a review of the published medical evidence of research and clinical trials in the National Library of Medicine, which were written up extensively in a previous published paper²¹.

Blood samples were taken at the beginning of the study and at the end of four months. Samples were processed according to the Elite Male and Elite Female Blood Test Panels, which included full chemistry, metabolic, lipid, complete blood count, cardiac markers and hormone panels, developed by Life Extension National Diagnostic Inc. For the chemistry blood analysis the specific focus was to measure changes in homocysteine (HCY), hemoglobin A1C (HgA1C), C-reactive protein (CRP), diabetic and coronary heart disease risk markers and serum vitamin D.

Cholesterol lipid markers were also tested at the beginning and at the completion of the four-month trial. Since the medical evidence regarding an association between high-density lipoprotein cholesterol (HDL-C) levels and memory deficit and cognitive function is not as well established as low-density lipoprotein cholesterol (LDL-C), the researchers were most interested in the latter. The large Honolulu-Asia Aging Study found that elevated LDL-C levels in midlife were associated with an increased risk of dementia and Alzheimer's disease in later life²². In addition, high LDL-C contributes to inflammation, oxidative stress and endothelial dysfunction, which have been implicated in adverse epigenetic DNA methylation modifications²³.

Separate blood serum samples were taken before and after for DNA methylation analysis based upon the DNAmGrimAge panels developed and processed by the Clock Foundation at the University of California Los Angeles. Every morning, a nurse measured everyone's pulse, temperature and, when necessary, blood sugar measurements of participants with a history of higher glucose levels. All participants were required to have regular impedance tests to measure muscle mass, bone mass and hydration levels.

Improvements in strength and stamina were measured before and after physical exercise tests.

The protocol to which participants adhered for the duration of the study prescribed regimens of diet, supplementation, physical exercise, intermittent fasting, stress-reduction and personal examination of emotions and beliefs. The following parameters comprise the protocol's parameters.

2.1. Diet

The prescribed diet was organic, vegan and gluten-free. A plant-based diet has been associated with lower risk of cardiovascular disease, heart attack, stroke, hypertension, diabetes and cancer^{24,25}. A gluten-free diet has been recognized as useful in treatment of celiac disease, gluten ataxia, dermatitis herpetiformis, cognitive impairment, inflammatory bowel disease and irritable bowel syndrome, non-celiac gluten sensitivity, fibromyalgia, endometriosis and chronic pelvic pain^{26,27}. Careful planning of a gluten-free diet was employed to avoid nutritional deficiencies²⁸. Consumption of organic foods versus conventional foods has certain nutritional advantages

including a reduction of exposure to pesticide residues and antibiotic-resistant bacteria^{29,30}.

The studies' diet was focused on phytonutrient rich fruits, vegetables, whole grains, legumes, seeds and nuts. The major daily meal was lunch followed by a light vegan meal for dinner, which comprised soups and salads. The diet specifically excluded refined carbohydrates, wheat and dairy, meat, poultry and fish. No caffeine, alcohol, refined sugar or artificial sweeteners or chemicals such as additives, preservatives, flavorings and no carbonated beverages were offered. The foods served at the retreat and which the participants were asked to eat on their return

Table 1: List of Daily Supplement Regimen.

Daily Supplement Regimen					
Vitamin A	1500 mcg				
Vitamin B Complex		Vitamin K2	2600 mcg	L-Carnosine	1000 mg
- Vitamin B1	75 mg	Omega-3		L-Ergothionine	5 mg
- Vitamin B2	1050 mcg	- DHA	510 mg	L-Taurine	800 mg
- Niacin	50 mg	- EPA	750 mg	L-Carnitine	500 mg
- Vitamin B6	75 mg	CoQ10	500 mg	Melatonin	2 mg
- Folate	680 mcg	Magnesium	100 mg	Resveratrol	55 mg
- Vitamin B12	300 mcg	Quercetin	264 mg	PQQ	10 mg
- Biotin	300 mcg	NAD+	300 mg	Bio-Fisetin	46 mg
- Pantothenic acid	50 mg	Zinc	25 mg	Curcumin	500 mg
- Inositol	50 mg	Selenium	200 mcg	Steviosides	375 mg
Vitamin C	500 mg	Chromium	200 mcg	Probiotic	74 mg
Vitamin D3	5000 IU	Iodine	150 mcg	Shilajit	50 mg
Vitamin E	67 mg	R Lipoic Acid	150 mg		

2.3. Juicing

Juicing fruits and vegetables removes the fibrous part of the plant and leaves the polyphenol rich juice which has been shown to improve the cardiovascular system, inhibit platelet aggregation, prevent hyper-homocysteinemia and possess anti-inflammatory, anti-oxidative, immune-modulatory and antimicrobial benefits to the gut microbiome and the body as a whole^{32,33}. To flood the body with these nutrients, the participants were required to drink twelve 8-oz vegetable and fruit juices per day, although juice was available for them at all times in case they wanted more. Juices were prepared according to a sequence of fruit juices in the morning and vegetable juices in the afternoon. This daily regimen was continued at home. All the participants were provided with juice recipes and received class instruction about the health benefits of juicing and how to prepare during their stay on-campus.

2.4. Exercise

Daily physical exercise is an essential lifestyle intervention associated with low all-cause mortality³⁴. Each participant followed an exercise and physical training schedule tailored to the level of each participant's specific health and physical requirements. Every morning, participants went for a power walk in nature before breakfast and would gradually increase their distance over the course of the study. Later in the day, a series of physical exercises practiced in the gym, which provided a full cardio-exercise program. Afternoon exercises included muscle resistance, water aerobics, biking, lateral pulls, sit-ups, push-ups, arm curls, squats, battling ropes and endurance exercises³⁵. A nurse onsite monitored participants' blood pressure and heart rate daily. Frequent measurements were taken to chart

home were all shown to be health-promoting and based upon peer-reviewed studies from the National Library of Medicine and included in the references³¹.

2.2. Nutritional Supplementation

Study participants followed the identical nutritional supplementation protocol of vitamins, minerals and bioactive nutraceuticals. The protocol was customized specifically for the study by the Life Extension Foundation. The following nutritional supplements and vitamins were consumed daily (Table 1).

the percent of body fat, muscle, weight, water retention and bone density. Before and after the retreat period, neck, chest, waist, hips, thighs, calves and biceps were measured and recorded.

2.5. Intermittent Fasting

Intermittent fasting has been shown to improve metabolic health and slow aging³⁶. Preserving cellular longevity requires substituting the presence of pathogenic micro-populations with healthy micro-cultures. Fasting aids in restoring gut health, regulates and lowers blood pressure by directly restructures the gut's microbiota that may regulate blood pressure and lessen the risk of diabetes^{37,38}. The participants were instructed to fast for a period of about fifteen hours, from evening (7 pm) until late morning (10 am) of the following day. Participants were permitted to have lemon water at 8 am prior to a morning power walking and exercise. The same fasting regimen was repeated throughout the remainder of the study at home. On weekends (Saturday and Sunday), only juicing was consumed and no solid foods.

2.6. Stress Reduction

Both physical and psychological stress are important factors implicated in the development and progression of disease and all-cause mortality³⁹. High psychological stress increases mortality risks among both those without pre-existing disease and those with multimorbidities, including heart disease and cancer⁴⁰⁻⁴². Stress reduction techniques have shown significant benefits for health. The study's protocol incorporated daily sessions in several stress reduction techniques: yoga, mindfulness meditation, Tai Chi Chuan, art therapy and private time in nature.

2.7. Counseling

Following the implications of stereotype embodiment theory, the study's protocol incorporated a daily educational and counseling parameter for participants to deal with negative emotions and to examine their inhibitory and harmful belief systems or mindsets. Professional counselors present on campus provided counseling to help them overcome psychological barriers to mental well-being. The participants also engaged in keeping a daily journal. Daily lectures delivered by the study's lead author Dr. Gary Null for participants were convened to improve individuals' states of mind on a variety of subjects including the psychological dynamics behind aging, happiness, anger, discovering purpose in life, love, gratitude, forgiveness and honoring one's strengths and weaknesses. They were also instructed on how to continue monitoring improvements in their physical endurance and stamina. Additional professional counselors on campus presented classes and oversaw the stress management program. All classes, lectures and workshops were held in silence (excluding the instructors), unless participants had questions to be answered or special needs that may arise. In addition, this trial relied heavily upon personal interaction between the lead author or an assistant with each participant to query about their subjective mental and emotional states. Regular personal engagement as means for psychological evaluation fills a blind spot often ignored in clinical trials since quantitative measurements alone do not properly evaluate the condition of the whole person in a reliable manner.

At the conclusion of the two weeks in residence at the retreat center, participants returned home to continue following the protocol to the best of their capabilities for the remaining 3 and half months (14 weeks) of the study. Twice a week, as a group they received additional classes and counseling live online via Zoom. These at-home group sessions also offered times for personal evaluation of psychological progress and improvement in general well-being. Since social isolation is a common habit among older adults, which can have detrimental psychological effects, participants were also encouraged to increase their oral social interactions. At the conclusion of the study, a final four-hour group gathering was conducted to evaluate each participants emotional improvement and reduction in stress, depression and anxiety.

3. Results

Since each of the 18 participants were screened to assure relatively good health with no current or prior serious illnesses, it was expected that there would be little or no substantial change in the blood markers evaluated. Upon entering the study, all participants had normal levels for Coronary Heart Disease Risk (<1.0) and homocysteine (<17.2). One of the 18 trial participants was deficient in serum vitamin D (<30) but this normalized at the end of the study. Seven of the 18 individuals had high C Reactive Protein (>3.0) and 8 of 18 had above normal hemoglobin A1c for determining glycemic control (>5.6). Six of the 8 individuals with high CRP showed lower levels at the conclusion of the study and 6 of these had levels decreasing to normal levels with the greatest reduction from 19.73 to 0.49. There was no significant change in hemoglobin A1c (p=0.157). Each participant had a normal HDL cholesterol level (>39) at the start of the study and no statistically significant changes were observed. Sixteen of the 18 participants started the study with high LDL-C levels (>99); 13 observed an LDL-C decrease and 3

reverted to normal levels. There was a statistically high decrease in total cholesterol (-12.8%, p=0.015) and more importantly a decrease in LDL cholesterol (-9.2%, p=0.025) (**Table 2**).

Table 2: Anti-Aging Blood Markers (4 months) N=18 participants (male = 10; female = 8).

Marker (mean)	Start Value	End Value	Change	% Change	P value <0.05
Est CHD Risk	0.59	0.56	-0.3	-3.59	0.110
Homocysteine	11.29	10.37	-0.92	-7.62	0.090
C Reactive Protein	4.45	2.83	-1.62	-27.82	0.316
Hemoglobin A1c	5.88	5.42	-0.46	-2.05	0.157
Vitamin D	54.09	49.45	-2.95	-9.45	0.454
Total Cholesterol	205.6	179.3	-26.3	-12.8	0.015
LDL Cholesterol	120.7	109.6	-11.1	-9.2	0.025

There were no significant observations for any adverse effects associated with oxidative stress in the blood markers or the DNAm results. Rather the DNAmFitAge results indicate an estimated 5.945-year biological age reduction calculated to chronological age with a 1.3-year standard error (p=0.0003). Hand-grip strength (DNAmGripAge) significantly increased when calculated without factoring in chronological age (p=0.00001). On average this added an estimated 0.58 years of grip strength to participants' lifespan. Although very small epigenetic improvements were noted in the longitudinal post treatment of DNAmGait or walking speed metric (no age p=0.932; with age p=0.416), nor in forced expiratory lung volume or DNAmFEV1 (p=0.907) there was no notably significant trend.

As expected, following daily brisk walking and muscle strength exercises, there was a significant increase in DNAmVO2max to measure maximal oxygen intake for cardiorespiratory fitness. On average there was an estimated 0.81 year (SE=0.24) increased lifespan (p=0.004) (**Table 3**).

Table 3: DNAmFitAge Epigenetic Clock Longitudinal Effects N=18 participants (male = 10; female = 8).

Marker	Estimate (years)	STD Error	P Value (<0.05)
DNAmFitAge	-5.945	1.32	0.0003
- DNAmGait (no age)	0.013	0.15	0.932
- DNAmGait (with age)	-0.042	0.05	0.416
- DNAmGrip (no age)	-0.576	0.07	0.00001
- DNAmGrip (with age)	0.098	0.03	0.007
- DNAmVO2max	0.807	0.24	0.004
- DNAmFEV1 (no age)	0.010	0.09	0.906
- DNAmFEV1 (with age)	0.142	0.08	0.075

Although there was small signs of positive methylation reversal in the DNAmAge (-1.582), DNAmGrim-Real (-1.603), DNAmGrim-Predicted (-1.731), DNAmGrim2-Real (-1.441) and DNAmGrim2-Predicted (-1.589), none of these markers displayed a statistically significant trend across the group with p values having been >0.05.

The physical exercise endurance metrics showed significant improvement across the group with only three individuals not showing insignificant improvement. The study's conclusion report showed mean endurance improvements in push-ups (134.9%), squats (107.5%), abdominal curl ups (246.7%), arm curls (72.5%) and leg lifts (74.5%). There was also a mean muscle gain of 30.4% and a 6.6% gain in bone density.

Finally, there was a very high improvement in every participant's final evaluation into their state of mind and sense of well-being, levels of psychological stress, depressed moods, anxiety, feelings of guilt, agitation, sleep disturbance and life satisfaction.

Despite these being subjective evaluations, the difference between their initial states of psychological health at the start of trial and at the trial's conclusion were substantial. Participants categorically discovered they had a sense of greater self-awareness, felt more vital, healthier and physically active and believed their lives were greatly optimized by having a new outlook on life.

4. Discussion

This study has shown that in a four-month period, older adults can substantially improve the quality of their physical endurance and stamina and their mental well-being with a comprehensive change in their lifestyles and habits that includes daily aerobic and muscle strength exercise, a plant-based diet, intermittent fasting, daily stress-reduction techniques and a positive change in their perceptions about themselves and their lives.

Each individual in the study engaged in physical aerobic and anaerobic exercises that were otherwise not a part of their daily lives. Exercise can have a dual effect on the immune system. Moderate exercise is generally associated with immune system enhancement, while excessive or prolonged exercise may suppress the immune response, increasing susceptibility to inflammation. Therefore, it was anticipated that there might be elevations in some of the blood markers associated with oxidative stress and inflammation at the cellular level. Increased oxygen consumption can lead to the production of reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals as mitochondrial byproducts during cellular respiration. ROS radicals can damage cellular structures including proteins, lipids and DNA. High levels of ROS can potentially overwhelm the body's antioxidant defenses.

The mechanical stress and micro-damage to tissues during intense exercise may trigger inflammatory responses as the body attempts to repair affected tissues. Intense exercise can also release pro-inflammatory cytokines such as interleukin-6 (IL-6) from macrophages, CD4+ T cells, CD8+ T cells and B cells and tumor necrosis factor-alpha (TNF- α) from macrophages, CD4+ T helper cells and natural killer (NK) cells. While these cytokines play a role in the body's response to stress and injury, their overproduction can contribute to chronic inflammation.

To counter the adverse effects of aerobic exercise, including oxidative stress and inflammation, which could adversely impact DNA methylation without implementing counter measures, the protocol's inclusion of several strategies such as dietary adjustments to increase anti-oxidative and anti-inflammatory nutrients, stress reduction techniques and lifestyle modification were implemented. The diet was rich in phytochemical antioxidants - fruits, vegetables, nuts and seeds - and specifically designed to provide overall health and resilience to the physiological demands of exercise. Daily juicing assured participants stayed well hydrated before, during and after exercise to support cellular function and maintain electrolyte balance. Post-exercise nutrition included necessary carbohydrates to replenish glycogen stores, protein to support muscle repair and growth and antioxidants to counter oxidative

stress. Daily supplement intake, notably of vitamin C, vitamin E, selenium, zinc, Omega-3 fatty acids and curcumin provided additional nutritional defense to mitigate inflammatory responses. Finally, daily stress reduction techniques such as mindfulness meditation, yoga and private personal time in nature to manage stress levels that can equally exacerbate inflammation. There was also adequate rest and recovery time between exercise sessions to enable the body to repair and adapt. These natural interventions very likely offset otherwise potential cellular aging as a result of the abrupt changes older adults were adopting over the course of the study.

This comprehensive approach for slowing the biological aging process likely contributed to the significant results in the GrimAge DNAm metrics for physical fitness longitudinal results in the DNAmFitAge parameters. In addition to improving cardiovascular health, the significant decrease in LDL-C, thereby reducing inflammation and oxidative stress, would have also created a more favorable environment for DNA repair observed in the DNAm results. The combination of lower LDL-C and a higher DNAmVO2max result would indicate a potential improvement in cognitive function and memory. During the final evaluation, the majority of the participants indicated they felt their cognitive abilities had improved.

However, McGreevy KM, et al. note that DNAm fitness biomarkers should not replace true fitness parameters. The DNAmFitAge markers rather identify the epigenetic changes that accompany physical fitness. Older adults who are physically fit have a younger DNAmFitAge. The study's limitation is the short 4-month time period undertaken. A 6-12 month trial, with a longer period of time conducted in an in-residence retreat environment, would have achieved better DNAm results in the other longitudinal parameters.

There was also inconsistency between the lifestyle regimen observed while in a group residence compared to continuing the protocol at home. Because each group spent the first two weeks of the study in a retreat setting with other participants and the study facilitators, there was a group dynamic that supported individual progress. Some individuals did not have easy access to gym facilities or open spaces for long distance power walking comparable to the retreat center nor physical fitness instructors to reinforce motivation. Several study members stated that their ability to continue the entire protocol at home daily was inconsistent and this may have contributed to lower results in their physical profile, endurance, blood test and DNA methylation results. Those who already led a relatively healthy lifestyle were more likely to adhere to this lifestyle modification regimen. Those who were most likely to not follow the protocol either did so with difficulty or had highly stressed lifestyles and unhealthy eating, daily activity and social habits at the start of the study. Consequently, this smaller latter group struggled more to make the radical adjustments during the study's short duration.

There is no consensual evidence that simply monitoring and measuring biological markers can alone account for increased emotional health and a person's sense of well-being and personal contentment. Taking this important factor out of the equation is counterproductive to the basic hypotheses of mind-body based medicine, which takes the psychological conditions into consideration. It is highly feasible that the inclusion of education and personal psychological counseling in self-motivation parameters, both as a group dynamic and

personalized to the individual, was a major contributor for the study participants to be able to make and sustain the radical changes in their daily behaviors and habits. Such an approach indicates that older adults are capable of sustaining a positive attitude about themselves and the aging process despite the negative stereotypes and their reinforcement held by the culture at large.

5. Conclusion

This observational study supports the efficacy of a comprehensive anti-aging protocol based upon complementary and integrative medical principles and therapies, including a plant-based diet, daily physical exercise, intermittent fasting, stress reduction and mind-body techniques and education and group counseling to reinforce healthy habits, constructive motivation and a change of perception about oneself to foment greater well-being and life satisfaction, to reverse biological aging markers in adults over 60 years of age. In particular, the protocol improved DNA methylation markers associated with physical fitness based upon DNAmFitAge, notably maximal oxygen intake for cardiorespiratory fitness. Other DNA methylation markers showed minor improvement, however, there was no consistent trend confirm their statistical significance. However, given the small decline in most participants' biological age according to the GrimAge scores, a follow up study extending beyond four months may very likely show statistically better results in DNAm aging markers. Finally, equally important was the improvement in quality of life and emotional well-being reported by each participant. The most notable subjective observation was the increased optimism towards living a more fulfilling life each participant had felt at the study's conclusion.

6. Data Availability

Data sets for the study are available from the corresponding author upon request.

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The research did not receive any funding from outside institutions in the public or private sectors.

8. Declaration of Competing Interest

The authors do not declare any competing financial interests

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There are no conflicts of interest among any of its authors.

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