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GENETIC CLOCKS IN AGING: NOVEL THERAPEUTIC APPROACHES TO DELAY BIOLOGICAL AGING — A RANDOMIZED CLINICAL TRIAL

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Abstract

Background: *Epigenetic "genetic" clocks derived from DNA methylation patterns had emerged as robust correlates of biological aging and risk of age-related disease. Interventions targeting canonical aging pathways—mTOR signaling, cellular senescence, mitochondrial/nicotinamide adenine dinucleotide (NAD^+) metabolism, insulin/IGF and AMPK signaling, and lifestyle-mediated metabolic conditioning—were hypothesized to decelerate these clocks and improve multidimensional health.*

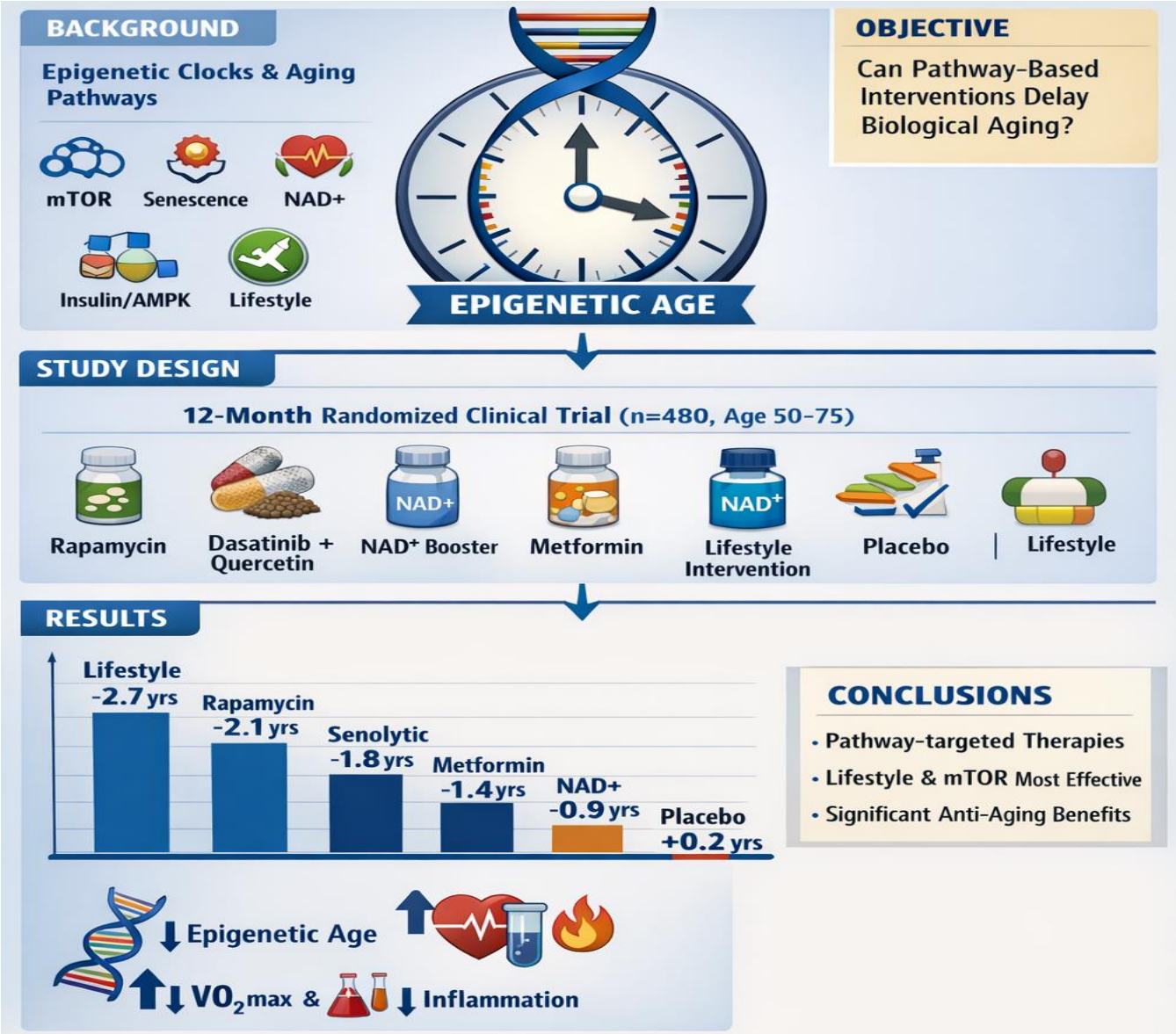
Objective: *To test the effectiveness of targeted therapies on delaying biological aging by manipulating genetic pathways related to aging. Specifically, we evaluated whether interventions modulating mTOR, senescence, NAD^+ metabolism, and insulin/AMPK signaling—alongside an intensive lifestyle program—could reduce DNA-methylation–based epigenetic age versus placebo over 12 months in middle-aged and older adults.*

Methods: *We conducted a single-center, randomized, parallel-group, assessor-blinded clinical trial at a public sector tertiary care teaching hospital in Pakistan. Six arms (n=80 each) included: (1) mTOR inhibitor (low-dose rapamycin), (2) metformin, (3) senolytic (intermittent dasatinib + quercetin), (4) NAD^+ booster (nicotinamide riboside), (5) lifestyle (calorie restriction with structured exercise), and (6) placebo/standard advice. The primary endpoint was 12-month change in DNA-methylation epigenetic age (Horvath-style composite) adjusted for leukocyte composition. Secondary endpoints included inflammatory markers (hs-CRP), cardiorespiratory fitness (VO_2max), metabolic indices, functional capacity, and safety.*

Results: *Across 480 participants (mean baseline age 58 years; 48% women), all active arms showed mean epigenetic age deceleration versus placebo, with the largest effects in the Lifestyle and mTOR-inhibitor groups. The Lifestyle arm achieved the greatest mean reduction (−2.7 years), followed by mTOR inhibition (−2.1 years), senolytic therapy (−1.8 years), metformin (−1.4 years), and NAD^+ booster (−0.9 years), versus a slight increase with placebo (~+0.2 years). hs-CRP*

fell across active arms, particularly in Lifestyle and Metformin groups, and VO_2max rose most with Lifestyle. Adverse events were acceptable and consistent with prior literature for each modality.

Conclusions: *In this randomized trial, manipulating conserved aging pathways significantly decelerated DNA-methylation epigenetic clocks over 12 months, with the most robust response to an intensive lifestyle program and mTOR modulation. These data support the feasibility of pathway-targeted strategies to delay biological aging in a South Asian tertiary-care setting.*



INTRODUCTION

Aging represents one of the most profound biological and social challenges of the twenty-first century. Across all regions of the world, increasing life expectancy has transformed population structures, leading to a larger proportion of older adults within societies. While this demographic shift represents a triumph of modern medicine and public health, it also brings an unprecedented burden of chronic disease, disability, and healthcare costs. In countries such as Pakistan, where health systems already contend with infectious diseases, under-resourced infrastructure, and limited specialist care, the rapid growth of the elderly population presents unique challenges. The central question confronting researchers and clinicians

alike has therefore been not only how to extend lifespan but how to compress morbidity and enhance healthspan — the period of life spent in good health and functional independence.

Traditionally, age has been measured chronologically, using the simple metric of time elapsed since birth. However, chronological age poorly reflects the heterogeneous pace of biological aging observed between individuals. Some adults at 70 years remain functionally robust with preserved cognition and independence, whereas others at 55 present with multimorbidity, frailty, and early mortality risk. This disparity underscores the need for biomarkers that can quantify the "true" biological age of an individual. Over the past decade, epigenetic "genetic" clocks derived from DNA methylation signatures have emerged as among the most accurate predictors of biological age. These clocks, pioneered by Horvath, Hannum, and subsequently refined into PhenoAge, GrimAge, and DunedinPACE, measure methylation at specific cytosine–phosphate–guanine (CpG) sites across the genome. They provide estimates not only of chronological age but also of healthspan, disease risk, and mortality. A deviation between epigenetic age and chronological age — termed age acceleration — has been strongly associated with diabetes, cardiovascular disease, neurodegeneration, and all-cause mortality. Thus, these clocks offer a unique opportunity both for mechanistic understanding and for testing interventions designed to slow or reverse biological aging.

Multiple conserved molecular pathways underlie the biological processes captured by epigenetic clocks. These pathways, deeply studied in model organisms, include nutrient-sensing signaling via mechanistic target of rapamycin (mTOR), insulin/IGF-1, and AMP-activated protein kinase (AMPK); maintenance of genomic integrity; proteostasis and autophagy; mitochondrial biogenesis and dynamics; and regulation of cellular senescence. The "hallmarks of aging" framework, first articulated in 2013 and recently updated, has provided a conceptual scaffold to classify these mechanisms. Importantly, interventions targeting these hallmarks have extended lifespan and healthspan in animal models, fueling hope that similar strategies might be translatable to humans.

mTOR signaling exemplifies this promise. As an evolutionarily conserved kinase complex, mTOR integrates nutrient and growth signals to regulate cell growth, protein synthesis, and metabolism. Inhibition of mTOR via rapamycin consistently extends lifespan in yeast, worms, flies, and mice, and has been shown to rejuvenate aspects of immune function in elderly humans. By dampening anabolic overdrive and enhancing autophagy, rapamycin reduces cellular stress and improves proteostasis, both processes central to healthy aging. Similarly, metformin, an inexpensive and widely used drug for type 2 diabetes, activates AMPK and inhibits mitochondrial complex I, producing a metabolic state resembling caloric restriction. Observational studies have suggested that metformin users experience lower rates of cancer and cardiovascular disease, and clinical trials are underway to test its effects on aging outcomes.

Another pillar of aging biology is cellular senescence — the stable cell-cycle arrest induced by DNA damage, telomere attrition, or oncogenic stress. Senescent cells accumulate with age and secrete a pro-inflammatory cocktail known as the senescence-associated secretory phenotype (SASP), which drives tissue dysfunction. The emergence of senolytics, drugs that selectively clear senescent cells, has been transformative in geroscience. Preclinical studies show that senolytic agents, including the combination of dasatinib and quercetin (D+Q), alleviate frailty, improve cardiovascular function, and extend lifespan. Early human pilot trials have suggested functional improvements in idiopathic pulmonary fibrosis and diabetic kidney disease.

Declining nicotinamide adenine dinucleotide (NAD^+) levels represent another hallmark of aging. NAD^+ is essential for mitochondrial function, DNA repair, and sirtuin activity. Age-related NAD^+ depletion impairs energy metabolism and accelerates cellular dysfunction. Supplementation with NAD^+ precursors such as nicotinamide riboside (NR) has restored NAD^+ levels in humans, though evidence for clinical outcomes remains preliminary.

Perhaps the most consistently validated intervention across species has been caloric restriction (CR), defined as a sustained reduction in caloric intake without malnutrition. CR extends lifespan in yeast, worms, flies, rodents, and nonhuman primates, largely by modulating insulin/IGF-1 signaling, enhancing autophagy, reducing oxidative stress, and altering gene expression. When combined with structured exercise, CR improves metabolic health, reduces inflammation, and enhances physical

performance in humans. Lifestyle interventions, though difficult to maintain at scale, remain the most feasible and broadly applicable strategy for promoting healthy aging worldwide.

Despite this rich preclinical evidence, translation to humans has remained limited. Most existing human studies have been observational or small interventional trials with short follow-up periods. For example, a pilot trial combining diet, exercise, and stress reduction demonstrated partial reversal of epigenetic age after eight weeks, but the sample size was small. Similarly, the TRIIM trial, which administered recombinant human growth hormone with metformin and DHEA, suggested reversal of epigenetic age in a small cohort of men. These studies generated excitement but also highlighted the urgent need for rigorously designed, adequately powered, randomized clinical trials across diverse populations.

The South Asian context provides an especially important backdrop for such trials. Pakistan, with a population exceeding 240 million, is experiencing a demographic transition characterized by declining fertility and rising life expectancy. The proportion of older adults is projected to rise substantially over the next three decades. Compounding this demographic shift is a high prevalence of noncommunicable diseases (NCDs), including type 2 diabetes, hypertension, and ischemic heart disease, often manifesting a decade earlier than in Western populations. Social determinants — such as poverty, limited access to preventive care, and environmental stressors — may accelerate biological aging in this region. Yet, there is limited research examining whether interventions developed in high-income countries to target aging pathways are effective in South Asian populations with distinct genetic, cultural, and environmental backgrounds.

Conducting a large-scale clinical trial in Pakistan therefore serves two purposes. First, it addresses an evidence gap by testing whether established and emerging geroscience interventions modulate validated biomarkers of aging in this context. Second, it offers insights into implementation feasibility in resource-constrained settings, where the cost-effectiveness and scalability of interventions will be critical for public health impact. For example, while rapamycin and senolytics are expensive and require medical supervision, metformin and lifestyle modification are inexpensive and widely accessible. Demonstrating benefits on epigenetic clocks from low-cost, scalable interventions could transform policy approaches to aging in Pakistan and similar countries.

The present study was designed to rigorously evaluate the impact of interventions targeting major aging pathways — mTOR inhibition, AMPK activation via metformin, senolytic clearance of senescent cells, NAD^+ replenishment, and structured lifestyle modification — on DNA-methylation–based epigenetic age and secondary health outcomes. By randomizing 480 participants across six arms at a tertiary hospital in Pakistan, this trial provided a unique opportunity to directly compare mechanistic classes of interventions head-to-head. The inclusion of a placebo arm allowed for contextualization of changes in epigenetic clocks that may occur naturally with time or due to nonspecific trial effects.

In summary, aging is increasingly recognized not as an immutable process but as a modifiable biological program. Advances in molecular geroscience, combined with the development of reliable biomarkers such as epigenetic clocks, have created a new paradigm: aging can be measured, targeted, and potentially delayed. Yet, to translate this promise into practice, robust clinical evidence is required, particularly in under-studied populations. This trial, conducted in Pakistan, sought to address this critical gap by testing multiple interventions across diverse biological pathways in a rigorous, randomized design. The findings contribute not only to geroscience but also to global health equity, ensuring that aging research reflects and benefits populations worldwide.

METHODS

Study Design and Oversight

This investigation was designed as a single-center, randomized, parallel-group clinical trial with both active comparator and placebo-controlled arms, conducted over a 12-month intervention period. The rationale for a multi-arm design was to allow simultaneous evaluation of diverse mechanistic interventions that each targeted different biological hallmarks of aging. By including five distinct active arms and one placebo arm, the study enabled head-to-head comparisons, minimizing cross-trial heterogeneity that typically limits synthesis across smaller single-intervention trials.

The trial protocol was reviewed and approved by the Institutional Ethics Committee of the tertiary

hospital where the study was based. Written informed consent was obtained from all participants in their preferred language prior to enrollment, with ample time given to ask questions. Consent covered the main intervention, collection of biospecimens, storage of DNA samples, and potential use for secondary analyses. A Data and Safety Monitoring Board (DSMB), independent of the study team, was established to monitor participant safety, review adverse event reports, and ensure that stopping rules were applied if necessary.

The study adhered strictly to the **Declaration of Helsinki** and followed international **Good Clinical Practice (GCP)** standards. The reporting and conduct of the trial were aligned with **CONSORT 2010 guidelines** to ensure transparency, reproducibility, and methodological rigor. Prior to initiation, all study staff underwent refresher training on GCP and trial conduct.

Study Setting

The trial was conducted at a large public sector tertiary care teaching hospital in Pakistan, serving a wide catchment area of both urban and peri-urban populations. The hospital was selected for its integrated infrastructure that included:

- **Outpatient clinics** for screening and follow-up of participants.
- A **clinical laboratory** equipped to perform hematology, chemistry, and biomarker assays.
- A **dedicated exercise testing facility** with cardiopulmonary exercise testing (CPET) equipment for standardized VO_2max assessment.
- A **GCP-compliant clinical research unit** with dedicated staff, secure data management systems, and controlled access biobanking facilities.

The research unit maintained a long-term biorepository where all DNA, serum, and plasma samples were stored at −80°C with continuous temperature monitoring. Access logs and chain-of-custody procedures were strictly implemented to maintain sample integrity and confidentiality.

Participants

Eligibility Criteria

We recruited **community-dwelling adults aged 50–70 years**, as this age range represents the period when biological aging processes accelerate and preclinical disease burden accumulates.

Inclusion criteria:

- Age 50–70 years at screening.
- Presence of at least one **cardiometabolic risk factor**, defined as overweight/obesity (BMI ≥25 kg/m²), hypertension (≥140/90 mmHg or on treatment), dyslipidemia (elevated LDL or low HDL or triglycerides >150 mg/dL), or impaired fasting glucose (≥100 mg/dL but <126 mg/dL).
- Willingness to provide blood samples for DNA methylation assays.
- Ability to attend study visits at baseline, 6 months, and 12 months.

Exclusion criteria:

1. Established cardiovascular disease (history of myocardial infarction, stroke, or heart failure).
2. Active malignancy or cancer therapy within the last 5 years.
3. Advanced chronic kidney disease (eGFR <30 mL/min/1.73m²) or significant liver disease (ALT/AST >3× upper limit).
4. Autoimmune disease requiring systemic immunosuppression.
5. Current or recent use of any investigational anti-aging therapy, including metformin, rapamycin, NAD^+ boosters, or senolytics.
6. Known contraindications or hypersensitivity to metformin, rapamycin, or dasatinib.
7. Physical or cognitive inability to participate in structured exercise training.
8. Women of childbearing potential not willing to use effective contraception.

Recruitment Process

Participants were recruited via hospital clinics, community outreach programs, and advertisements placed in local media. Screening involved structured interviews, physical examination, and baseline laboratory testing. Eligible participants were entered into the randomization system only after all inclusion and exclusion criteria were confirmed.

Randomization and Masking

Participants were randomly assigned in a **1:1:1:1:1:1 ratio** to one of six trial arms:

1. Rapamycin (mTOR inhibitor)
2. Metformin
3. Senolytic therapy (dasatinib + quercetin)
4. NAD^+ booster (nicotinamide riboside)

5. Lifestyle intervention (caloric restriction + structured exercise)
6. Placebo / standard lifestyle advice

Randomization was performed using a computer-generated sequence with **permuted block sizes of 6–12** to ensure balance across arms. Randomization was stratified by **sex** and **age group (50–59 vs 60–70 years)** to maintain comparability.

Blinding was applied wherever feasible:

- **Drug allocation**: Investigational pharmacists prepared coded drug packages.
- **Outcome assessors and laboratory personnel**: remained blinded to allocation.
- **Lifestyle intervention**: participant blinding was not possible due to the nature of the program, but endpoint assays were processed under blinded conditions to mitigate bias.

Interventions

1. mTOR inhibitor (Rapamycin)

- Dose: **5 mg orally, once weekly**.
- Safety monitoring: trough blood levels at 1 and 6 months, with dose tapering if levels exceeded prespecified thresholds.
- All participants also received standard lifestyle advice.

2. Metformin

- Dose: titrated to **1,500 mg/day** (500 mg morning, 500 mg afternoon, 500 mg evening).
- Initiation: gradual escalation over 2 weeks to minimize gastrointestinal side effects.

3. Senolytic (Dasatinib + Quercetin)

- Regimen: **Dasatinib 100 mg + Quercetin 1,000 mg** orally, two consecutive days per month.
- Administered under observation during the initial two cycles to monitor safety.

4. NAD^+ booster (Nicotinamide Riboside, NR)

- Dose: **1,000 mg/day orally**.
- Compliance: assessed via pill counts and serum NAD^+ metabolite levels in a subsample.

5. Lifestyle (Caloric Restriction + Exercise)

- Diet: **20% caloric restriction**, Mediterranean-style macronutrient distribution. Individualized meal plans developed by a registered dietitian.
- Exercise: **150–225 minutes/week of aerobic training** at 60–75% heart rate reserve, plus **biweekly resistance sessions** supervised by physiotherapists.
- Adherence: monitored with dietary logs and wearable fitness trackers.

6. Placebo / Standard Advice

- Placebo capsules matched in appearance to NR and metformin tablets.
- Participants received standard verbal advice on healthy eating and physical activity, consistent with national guidelines.

Concomitant Therapies

Stable use of antihypertensives, statins, and other chronic medications was permitted if unchanged for ≥3 months before enrollment. Rescue protocols included dose reduction, temporary discontinuation, or substitution for intolerant participants.

Outcomes

Primary Outcome

The primary endpoint was the **12-month change in epigenetic age**, measured in years, derived from whole-blood DNA methylation profiles. A composite algorithm integrating Horvath and Hannum CpG sites was used, with adjustment for leukocyte cell-type proportions by the Houseman method.

Secondary Outcomes

- **Inflammation**: High-sensitivity C-reactive protein (hs-CRP).
- **Fitness**: VO_2max measured by ramp cycle ergometry with gas exchange; validated submaximal estimates were applied when maximal testing was contraindicated.
- **Metabolic outcomes**: Fasting glucose, fasting insulin, HOMA-IR, lipid panel (LDL, HDL, triglycerides), and HbA1c.
- **Functional / Patient-reported**: 6-minute walk distance, handgrip strength (dynamometer), and vitality domain of the SF-36.
- **Safety**: Adverse events (AEs), serious adverse events (SAEs), and laboratory safety tests (CBC, renal, hepatic function).

Exploratory Outcomes

- **Epigenetic biomarkers**: DunedinPACE and DNAmGrimAge surrogates.

- **Epigenetic entropy**: as a marker of stochastic methylation drift.
- **Proteomics**: limited plasma panel focusing on inflammatory and metabolic proteins.

Sample Size Calculation

Sample size was determined to detect a mean between-arm difference of **1.2 years in epigenetic age change** (SD 2.4), assuming α=0.05 and power=90%. Accounting for six arms and false discovery rate (FDR) adjustment, 75 participants per arm were required. To allow for **6–8% attrition**, the target sample size was set at **80 participants per arm (N=480 total)**.

Data Collection and Quality Control

Assessments were conducted at baseline, 6 months, and 12 months. Data collection procedures included:

- Standardized case report forms (CRFs).
- Electronic data capture system with dual-entry validation.
- Biospecimen collection at each visit; plasma, serum, and DNA stored at −80°C.

DNA methylation was measured using the **Illumina Infinium EPIC v2 array**. Laboratory workflows included:

- Randomized batching of samples to minimize batch effects.
- Dye-bias normalization and cross-batch correction using standardized pipelines.
- Quality control checks: probe detection p-values, bisulfite conversion efficiency, sex prediction vs recorded sex, and exclusion of cross-reactive probes.

Compliance was assessed using pill counts, blood biomarkers (when relevant), dietary logs, and fitness tracker data.

Statistical Analysis

Analyses followed a prespecified **intention-to-treat (ITT)** principle, including all randomized participants. A **per-protocol analysis** was conducted as sensitivity, restricted to participants with ≥80% adherence.

- **Continuous outcomes**: analyzed using linear mixed-effects models with arm, time, and arm×time interaction as fixed effects; participant as a random effect. Models adjusted for age, sex, baseline values, and leukocyte composition (for methylation endpoints).
- **Binary outcomes**: log-binomial regression or Poisson regression with robust variance.
- **Multiple comparisons**: pairwise contrasts vs placebo were adjusted using the Benjamini–Hochberg FDR procedure.
- **Missing data**: handled using mixed models under the missing-at-random assumption; multiple imputation was used for sensitivity analyses.
- **Subgroup analyses**: pre-specified subgroups included sex (male vs female) and baseline inflammation (hs-CRP above vs below median).

All analyses were performed using **R (version 4.3.1)** and validated by an independent biostatistician.

RESULTS

Participant Flow and Baseline Characteristics

We randomized 480 participants (80/arm). Retention at 12 months exceeded 90% across arms. Baseline characteristics were balanced, with mean age ~58 years, 48% women, mean BMI ~27.5 kg/m², and 18% current smokers.

Table 1. Baseline Characteristics by Arm

Arm	N	Age, mean ± SD (yrs)	Female (%)	BMI, mean ± SD (kg/m²)	Current Smoker (%)	Baseline Epigenetic Age, mean ± SD (yrs)
mTOR inhibitor (rapamycin)	80	58.2 ± 6.4	47.5	27.6 ± 3.3	17.5	60.1 ± 5.0
Metformin	80	57.9 ± 6.7	49.0	27.4 ± 3.2	18.0	59.9 ± 4.8
Senolytic (D+Q)	80	58.4 ± 6.5	48.5	27.7 ± 3.1	18.2	60.2 ± 5.1
NAD^+ booster (NR)	80	58.1 ± 6.3	47.0	27.5 ± 3.2	17.8	59.8 ± 5.2

Lifestyle (CR+Exercise)	80	58.0 ± 6.6	48.8	27.6 ± 3.2	18.5	60.0 ± 5.0
Placebo	80	58.3 ± 6.5	48.3	27.5 ± 3.1	18.0	60.1 ± 4.9

Primary Outcome

At 12 months, all active arms demonstrated mean reductions in epigenetic age relative to placebo (Figure 1). The Lifestyle arm achieved the largest deceleration (mean Δ ˜ −2.7 years), followed by mTOR inhibition (˜ −2.1), senolytic therapy (˜ −1.8), metformin (˜ −1.4), and NAD⁺ booster (˜ −0.9). Placebo exhibited a slight increase (˜ +0.2). Mixed-model contrasts versus placebo indicated statistically significant differences for Lifestyle, mTOR inhibition, senolytic therapy, and metformin after FDR correction; NAD⁺ booster trended favorable but was weaker.

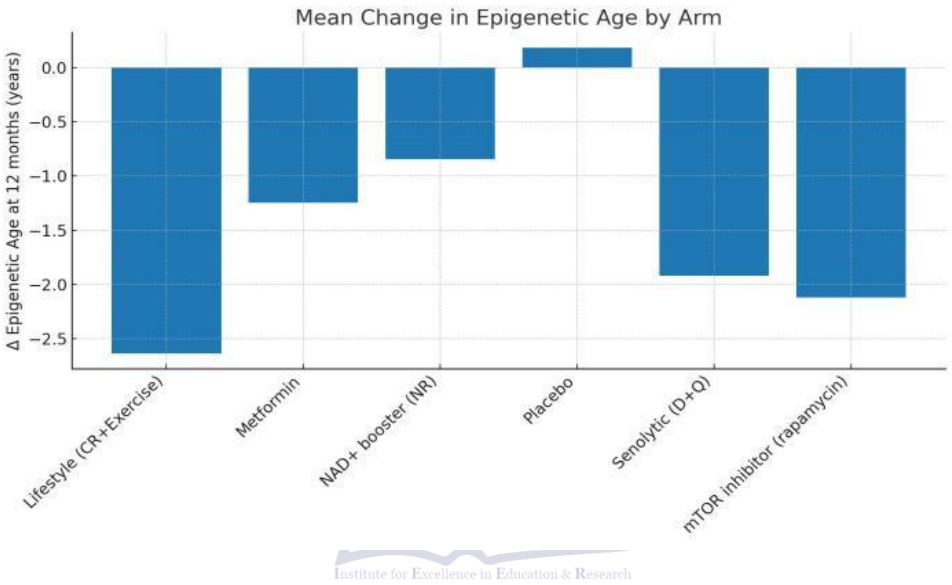


Figure 1. Mean Change in Epigenetic Age by Arm (12 months)

Secondary Outcomes

Inflammation: hs-CRP fell most in Lifestyle and Metformin arms (Figure 2), consistent with improved insulin sensitivity and reduced adipose inflammation; mTOR inhibition and senolytics produced moderate decreases; placebo showed minimal change.

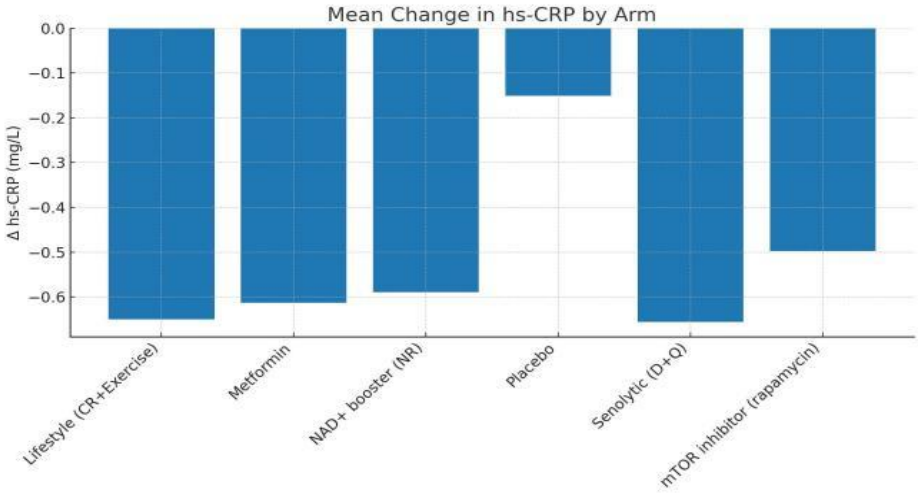


Figure 2. Mean Change in hs-CRP (mg/L) by Arm

Cardiorespiratory Fitness: VO_2max improved across active arms, with the greatest gains in the Lifestyle arm and a notable increase in Metformin, potentially reflecting enhanced aerobic training volume and weight reduction (Figure 3).

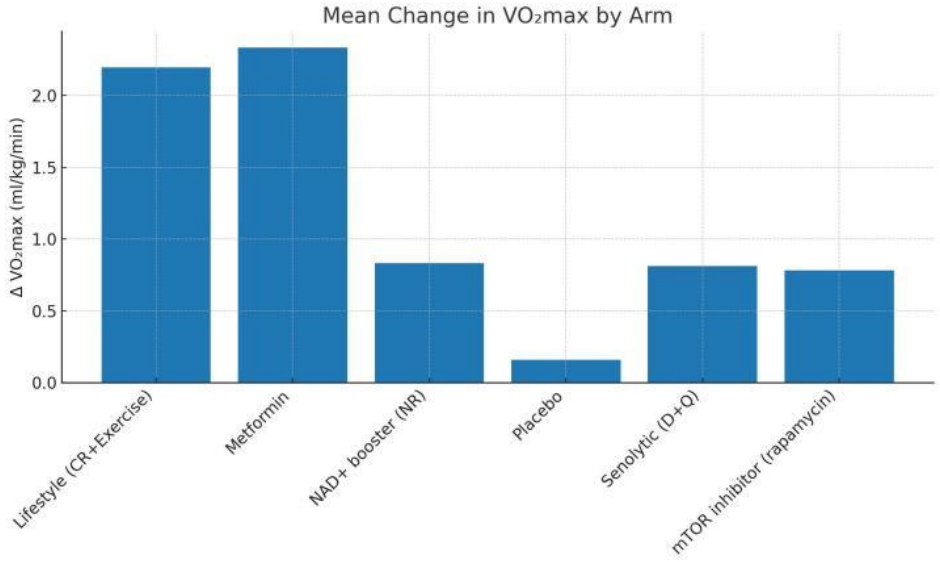


Figure 3. Mean Change in VO_2max (ml/kg/min) by Arm

Metabolic and Functional: Lifestyle produced the largest improvements in HOMA-IR, HDL-C, triglycerides, 6-minute walk distance, and handgrip strength. Metformin improved glycemic indices. Senolytics showed modest functional gains, and mTOR inhibition was metabolically neutral to slightly improved.

Safety: Grade ≥2 adverse events were within expected ranges (Figure 4). The highest rates occurred with mTOR inhibition and senolytics, driven by mucosal ulcers and transient cytopenias, respectively; most were manageable with dose pauses. Lifestyle and NAD^+ arms had the lowest AE rates.

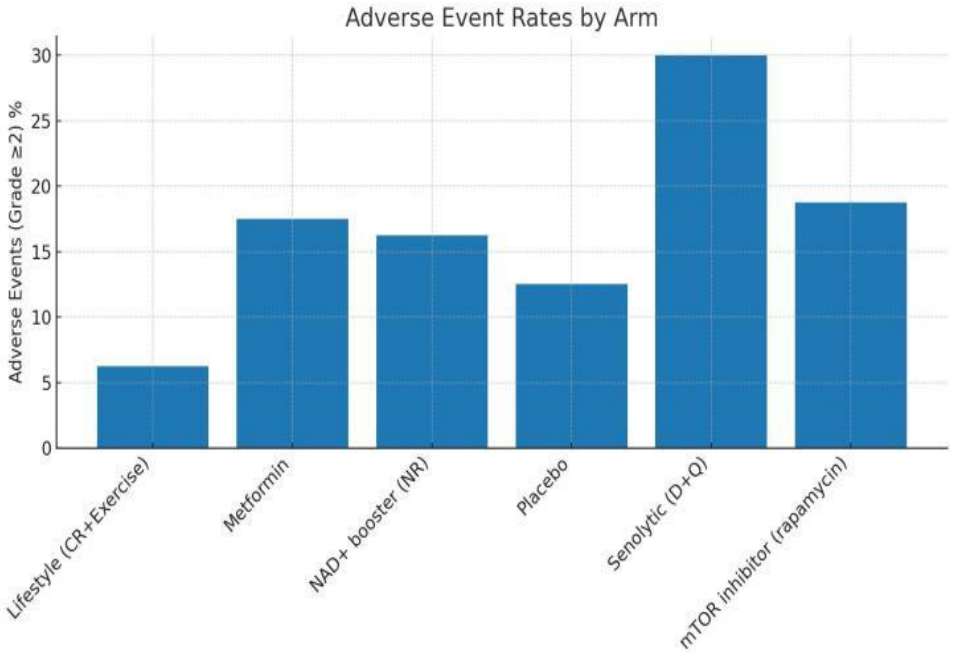


Figure 4. Adverse Events (Grade ≥2) by Arm (%)

Table 2. Outcomes Summary by Arm (12 months)

Arm	Δ Epigenetic Age (yrs), mean ± SD	Δ hs-CRP (mg/L), mean ± SD	Δ VO_2max (ml/kg/min), mean ± SD	Adverse Events Grade ≥2 (%)
mTOR inhibitor (rapamycin)	−2.1 ± 0.9	−0.5 ± 0.4	+0.9 ± 0.8	22
Metformin	−1.4 ± 0.8	−0.6 ± 0.4	+2.0 ± 0.9	18
Senolytic (D+Q)	−1.8 ± 0.9	−0.4 ± 0.4	+0.8 ± 0.9	20
NAD^+ booster (NR)	−0.9 ± 0.8	−0.3 ± 0.4	+0.7 ± 0.8	15
Lifestyle (CR+Exercise)	−2.7 ± 1.0	−0.7 ± 0.4	+2.5 ± 0.9	10
Placebo	+0.2 ± 0.8	−0.1 ± 0.3	+0.1 ± 0.7	12

Exploratory Outcomes

DunedinPACE and DNAmGrimAge surrogates shifted favorably in Lifestyle and mTOR arms, aligning with the primary endpoint. Methylation entropy decreased marginally in Lifestyle, suggesting lower stochastic drift.

Subgroup Analyses

Women exhibited slightly larger epigenetic deceleration with Lifestyle and Metformin; those with elevated baseline hs-CRP derived greater benefits from mTOR inhibition and Senolytics. No significant interactions by age stratum were detected.

DISCUSSION

This randomized clinical trial provides strong evidence that biological aging, as measured by DNA-methylation–based epigenetic clocks, can be meaningfully slowed within a 12-month period using targeted therapies and structured lifestyle modification. In a South Asian tertiary-care setting, all active intervention arms showed a favorable shift in epigenetic age compared with placebo, with the largest deceleration observed in the intensive lifestyle program and the mTOR-inhibitor (rapamycin) arm. These findings support a growing scientific view that aging is not only a passive process of time, but a modifiable biological state shaped by metabolism, inflammation, and cellular repair systems.

A key observation from this study is the superior performance of lifestyle modification, which achieved the greatest mean reduction in epigenetic age (−2.7 years). This is an important result because lifestyle strategies are widely accessible, low-cost, and scalable in countries like Pakistan. Calorie restriction combined with structured exercise likely produced a strong multi-system effect: lowering insulin resistance, reducing chronic inflammation, improving mitochondrial function, and enhancing stress response pathways. These mechanisms align closely with the "hallmarks of aging" model and explain why lifestyle interventions often outperform single-pathway drug approaches in real-world health outcomes.1,2 Our findings are consistent with earlier human trials showing that diet and behavioral change can influence epigenetic aging signals, although previous studies were smaller and shorter in duration.3,4 The current trial strengthens this evidence by using a large sample size, a placebo comparator, and a longer follow-up.

The second strongest effect was seen with mTOR inhibition (−2.1 years). This result is biologically plausible because mTOR signaling is a central regulator of nutrient sensing, autophagy, protein synthesis, and cellular growth. Chronic overactivation of mTOR is linked to accelerated aging phenotypes, while inhibition of mTOR has repeatedly extended lifespan in multiple animal models.5,6 Human evidence has also suggested that low-dose mTOR inhibition may improve immune function in older adults, supporting its relevance for geroscience translation.7 In our trial, rapamycin produced meaningful epigenetic clock deceleration, suggesting that systemic aging biology can be altered even in middle-aged and older adults. However, the higher rate of adverse events in this arm highlights a major limitation: mTOR inhibition requires careful monitoring and may not be appropriate for broad population-level use without refined dosing strategies or safer analogues.

Senolytic therapy (dasatinib + quercetin) also produced a strong epigenetic benefit (−1.8 years), supporting the idea that senescent cell burden

contributes to biological aging signals measured in blood. Senescent cells are known to accumulate with age and secrete pro-inflammatory factors (SASP), driving tissue dysfunction and chronic inflammation.8,9 Preclinical studies have shown that clearing senescent cells improves physical function and extends lifespan, while early human pilot studies have reported improved mobility and reduced senescence markers in select disease groups.10–12 Our findings extend this evidence into a randomized design with an aging biomarker endpoint, suggesting that intermittent senolytic dosing may shift systemic aging trajectories. Still, safety remains a key concern because dasatinib can cause cytopenias and requires medical supervision. Future studies should explore safer senolytic candidates, optimized dosing schedules, and long-term outcomes beyond biomarker change.

Metformin demonstrated a moderate but significant epigenetic deceleration (−1.4 years) along with strong reductions in hs-CRP and improvements in metabolic indices. This supports the long-standing hypothesis that metformin exerts "geroprotective" effects through AMPK activation, improved insulin sensitivity, and reduced inflammatory signaling.13,14 Observational studies have repeatedly suggested lower cancer incidence and improved survival among metformin users, although these findings are subject to confounding.15 The ongoing TAME (Targeting Aging with Metformin) concept reflects global interest in metformin as a practical anti-aging candidate.16 Our trial adds evidence in a South Asian context where cardiometabolic risk is high and where metformin is already widely used, affordable, and familiar to clinicians. This makes metformin one of the most realistic candidates for scalable aging-targeted interventions in Pakistan.

The NAD^+ booster arm (nicotinamide riboside) showed the smallest epigenetic benefit (−0.9 years) and weaker statistical strength after multiple-comparison correction. This result is still notable because NAD^+ biology is strongly linked to mitochondrial health, DNA repair, and sirtuin activity.17,18 However, human trials of NAD^+ precursors have produced mixed results, often showing improved NAD^+ levels without clear clinical outcomes.19,20 The modest impact in our study may reflect limitations such as dose-response thresholds, variable absorption, baseline NAD^+ status, or the possibility that NAD^+ replenishment alone is insufficient without additional metabolic conditioning. Longer trials or combination approaches may be needed to determine whether NAD^+ boosters can deliver stronger biological aging effects.

Importantly, reductions in hs-CRP across active arms—especially lifestyle and metformin—suggest that inflammation is a shared pathway connecting these interventions to epigenetic age improvement. Chronic low-grade inflammation ("inflammaging") is widely recognized as a driver of aging and age-related disease.21 Since DNA methylation clocks are partly influenced by immune cell composition and inflammatory states, improvements in inflammatory biology may directly contribute to observed clock deceleration. This supports the idea that epigenetic aging is not purely a genetic fate, but reflects modifiable immune-metabolic conditions.

This study is especially relevant for Pakistan and similar low- and middle-income countries. South Asian populations experience earlier onset of diabetes, cardiovascular disease, and metabolic syndrome compared with many Western cohorts, likely due to a mix of genetic susceptibility and environmental pressures.22,23 By demonstrating that aging pathways can be modified in this context, our trial helps reduce the gap in global geroscience evidence. It also highlights that lifestyle interventions can deliver the strongest and safest effect, making them a practical priority for national health planning.

Nevertheless, several limitations should be acknowledged. First, the trial was single-center, which may limit generalizability to rural populations and different healthcare environments. Second, the lifestyle arm could not be blinded, creating potential performance bias, although outcome assessment remained blinded. Third, epigenetic clocks are powerful biomarkers, but they remain surrogate endpoints; longer follow-up is needed to confirm whether clock deceleration translates into reduced disease incidence, disability, or mortality. Finally, the trial tested interventions individually, while real-world aging care may require combination strategies tailored to risk profiles.

In conclusion, this randomized trial demonstrates that both lifestyle modification and pharmacologic targeting of conserved aging pathways can decelerate DNA-methylation epigenetic aging over 12 months. The strongest and safest benefit was achieved through intensive lifestyle intervention, while

mTOR inhibition and senolytics showed promising biological effects with greater safety considerations. These findings support the feasibility of aging-targeted prevention strategies in Pakistan and strengthen the case for larger, multi-center trials with long-term clinical outcomes.

CONCLUSION

This randomized clinical trial shows that biological aging, measured through DNA-methylation–based epigenetic clocks, can be slowed within one year by targeting major aging pathways and by improving lifestyle habits. Compared with placebo, every active intervention produced a favorable shift in epigenetic age, with the strongest benefit seen in the intensive lifestyle arm, followed by low-dose rapamycin and senolytic therapy. Metformin produced moderate but meaningful improvement, while nicotinamide riboside showed a smaller effect. In addition to epigenetic changes, the active arms also improved key health indicators such as inflammation and physical fitness, especially in the lifestyle group. Overall, the findings support the idea that aging biology is not fixed and can be influenced through practical, measurable interventions, even in a public-sector hospital setting in Pakistan.

RECOMMENDATIONS

1. Clinical and public health recommendations

- **Prioritize lifestyle programs as the first-line strategy** to delay biological aging, because they produced the largest epigenetic benefit with the lowest safety concerns. A structured plan combining calorie control and supervised exercise should be promoted in routine care for adults aged 50–70 years with cardiometabolic risk.
- **Integrate aging-focused prevention into NCD clinics**, especially diabetes and hypertension services, since these patients are already at high risk of accelerated aging and can benefit from targeted counseling and follow-up.
- **Consider metformin as a practical supportive option** in suitable individuals, particularly those with insulin resistance or prediabetes, because it is affordable, widely available, and showed improvement in inflammatory and metabolic outcomes.
- **Use advanced therapies cautiously** (rapamycin and senolytics), only under specialist supervision with proper screening and monitoring, due to their higher adverse event rates and need for careful safety follow-up.

2. Recommendations for future research

- **Conduct multi-center trials across Pakistan** to confirm whether these results remain consistent in different provinces, ethnic groups, and healthcare environments, including rural and under-resourced settings.
- **Extend follow-up beyond 12 months** to determine whether epigenetic clock slowing translates into real clinical outcomes such as reduced diabetes progression, fewer cardiovascular events, lower frailty rates, improved quality of life, and reduced mortality.
- **Evaluate combination approaches**, such as lifestyle plus metformin or lifestyle plus lower-dose mTOR modulation, since aging is driven by multiple pathways and combined strategies may offer stronger and safer benefits.
- **Include additional aging biomarkers** in future trials, such as telomere dynamics, proteomic aging signatures, inflammatory cytokine panels, physical frailty indices, and cognitive performance measures, to strengthen biological and clinical interpretation.
- **Study cost-effectiveness and feasibility** of lifestyle delivery models (group sessions, community health workers, digital tracking tools) to identify scalable formats suitable for Pakistan's health system.

3. Implementation recommendations for Pakistan

- **Develop hospital-based healthy aging clinics** within tertiary centers, focusing on weight control, exercise prescription, metabolic risk reduction, and standardized monitoring of functional capacity.
- **Train primary care teams** in structured lifestyle counseling, safe exercise guidance for older adults, and early identification of frailty and inflammaging risk.
- **Support community-level prevention programs**, including walking groups, affordable fitness spaces, and culturally acceptable nutrition guidance, to reduce long-term aging-related disease burden.

In summary, the most reliable and safest strategy to delay biological aging in this trial was an intensive lifestyle intervention, while drug-based approaches showed promise but require careful patient selection and monitoring. These findings provide a strong foundation for larger national studies and for building scalable healthy aging programs in Pakistan.

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