Review

Epigenetics of Aging in Mammals: Mechanistic Foundations and Intervention Effects on DNA Methylation–Based Aging Biomarkers

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Epigenetics of Aging in Mammals: Mechanistic Foundations and Intervention Effects on DNA Methylation–Based Aging Biomarkers

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Abstract

Background: Aging is shaped by interdependent molecular processes captured by the hallmarks framework, in which epigenetic alterations stand out as a potentially modifiable regulatory layer.[1] DNA methylation (DNAm) patterns change with age and can be summarized by epigenetic clocks that estimate biological age, pace of aging, and risk-related phenotypes.[8,9,11-13] Yet, the extent to which interventions reproducibly modulate DNAm-based biomarkers across tissues and species remains uncertain.[6,8] **Methods:** A systematized review of longitudinal intervention studies (2010–2025; English/Spanish) was conducted in PubMed, Scopus, and Cochrane CENTRAL, with selection documented using PRISMA. Human eligibility included randomized controlled trials (RCTs), non-randomized controlled studies, and pre–post designs (n≥10; adults ≥18 years). Preclinical eligibility included longitudinal mammalian studies (n≥5 per group). Outcomes were changes in DNAm-based epigenetic age (years) and/or pace of aging (e.g., DunedinPACE). Data were extracted into a standardized matrix (clock, tissue, effect direction/magnitude, safety, RoB_overall) and synthesized narratively; meta-analysis was not performed due to heterogeneity. **Results:** Thirty-five longitudinal studies were included (29 human, 6 preclinical). Lifestyle interventions in humans generally showed modest effects,[14-26] with more consistent signals when exposure was sustained and accompanied by plausible physiological changes (e.g., prolonged calorie restriction affecting DunedinPACE, with effect sizes up to d=−0.43 at 12 months and d=−0.40 at 24 months in higher-adherence participants).[20] Exogenous compounds showed higher heterogeneity and mixed evidence,[27-41] including robust null epigenetic findings in some trials (e.g., metformin adjusted ITT differences ranging from −0.91 to +0.82 years across clocks, all p≥0.18)[27] alongside favorable signals in smaller analytic subsets or open-label settings (e.g., bezisterim sub-study with reductions of −3.68 years in SkinBloodAge, −5.00 in Hannum, and −4.77 in InflammAge).[28] Blood/circulation-derived interventions produced some of the largest reported effect sizes but also raised interpretation challenges: therapeutic plasma exchange with a sham arm reported epigenetic age decreases of ~1.3–2.6 years depending on the clock and regimen, with pronounced shifts in immune/inflammation-sensitive clocks; the apparent benefits waned after treatment cessation.[42] Unexpectedly, repeated plasmapheresis in donors was associated with increases in several clocks and DunedinPACE per procedure (~+0.16–0.26 years per session across GrimAge-family clocks and ~0.003±0.001 DunedinPACE units per session).[43] In rodents, plasma fractions/exosome-rich preparations and heterochronic parabiosis reported large percentage reductions across tissues, with strong dependence on exposure duration and concerns about translational uncertainty (up to ~77.6% in liver and ~68.2% in blood in one plasma-fraction study).[45-47] Evidence for partial reprogramming (OSKM) was limited to a single rat study with small, near-significant trends in hippocampus-based clocks (two-sided p=0.064–0.088 across three clocks).[48] **Conclusions:** DNAm-based epigenetic biomarkers are modifiable by interventions in mammals, but effects are heterogeneous and depend on the intervention, clock construct (age vs pace/risk signatures), biological matrix, tissue, follow-up duration, and study design. A single notion of "epigenetic rejuvenation" is not supported; instead, intervention effects appear domain-specific and must be interpreted in relation to what each clock measures.

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Introduction

Aging can be conceptualized as a network of interdependent cellular and molecular processes that collectively drive progressive functional decline and increased disease risk.[1] Since the first hallmarks of aging framework was first introduced in 2013, proposing nine interconnected hallmarks organized into primary, antagonistic, and integrative categories[2], this model has been expanded to encompass twelve interrelated hallmarks.[1] Within this network, epigenetic alterations stand out for their regulatory reach and relative reversibility.[1,3,4]

There is increasing consensus that epigenetic changes—DNA methylation, histone modifications, and dysregulation of non-coding RNAs—are integral to aging.[3-6] These changes can directly alter gene expression, compromise genomic stability, and promote retroelement reactivation, thereby shaping cellular phenotypes that propagate to organism-level function.[3-6] At the same time, the question of causality versus correlation remains unresolved for many age-associated epigenetic changes, compounded by tissue-specificity and crosstalk with other hallmarks.[3,6-8]

To address this challenge, DNA methylation–based epigenetic clocks have emerged as practical tools for quantifying biological aging, capturing biological age, pace of aging, and related outcomes such as mortality risk.[7.10] Over time, these measures have diversified to include multi-tissue and blood-based first-generation clocks (e.g., Horvath, Hannum)[8,11], second-generation clocks trained on clinical risk phenotypes (e.g., PhenoAge, GrimAge/GrimAge2),[12,13] and pace-of-aging measures such as DunedinPACE.[9] These tools have catalyzed a rapidly expanding intervention literature seeking to test whether biological aging signatures are modifiable.[3,4]

Beyond measurement lies translation. Although numerous geroprotective strategies have been proposed, the extent to which interventions reproducibly and meaningfully modulate epigenetic clocks remains limited and heterogeneous across designs, tissues, and clocks.[6,8] This systematized review therefore focuses on longitudinal intervention evidence in mammals, aiming to map which intervention families show the most consistent and interpretable effects on DNAm-based biomarkers, under what conditions, and with what safety considerations.

Methods

Review Design and Question

This work synthesizes longitudinal evidence on interventions that modulate epigenetic mechanisms and their effects on DNAm-based epigenetic age and/or pace of aging in mammals. The intervention-focused component was conducted as a systematized review with study selection reported using a PRISMA flow diagram.

PICO question: In adult mammals (humans and animal models), do interventions that modulate epigenetic mechanisms reduce epigenetic age and/or pace of aging compared with placebo/standard care or relative to baseline (pre–post)?

Eligibility Criteria

Criteria applied to the intervention review.

Population: Adult mammals. Humans (primary stratum): adults ≥18 years, healthy or with age-related conditions. Preclinical stratum: mouse or other mammals, n≥5 per group (one preclinical study with n=4 per group was retained as an exception due to its relevance).

Interventions: Interventions reporting longitudinal DNAm-based outcomes (epigenetic age and/or pace of aging). Exclusively in vitro interventions were excluded.

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Comparators: Placebo/standard care (parallel comparison) or within-subject pre–post comparison.

Outcomes: Primary: (1) change in DNAm-based epigenetic age (years) using methylation clocks (e.g., Horvath, Hannum, PhenoAge, GrimAge); (2) change in pace of aging (e.g., DunedinPACE or equivalent measures when applicable).

Secondary: safety and adverse events when reported.

Exclusions: In vitro studies, non-mammals, purely cross-sectional designs, sample size below thresholds, duplicates, editorials/reviews, lack of full text, or absence of epigenetic outcomes.

Information sources and search strategy

Databases: PubMed, Scopus, and Cochrane CENTRAL.

Time frame: 2010–2025. Languages: English/Spanish.

Last search date for the intervention review: 22/11/2025.

Search strings combined epigenetic clock terms (e.g., "epigenetic clock", "DNAm age", Horvath, Hannum, PhenoAge, GrimAge, DunedinPACE) with intervention terms. Full Boolean strategies and filters are provided in Appendix A.

Table 1. Full search strategies by database.

ID	Database	Date	Query	Filters	Results	Exported	Selected
I1	PubMed (humans)	21/11/2025	("epigenetic clock"[tiab] OR "epigenetic clocks"[tiab] OR "DNA methylation age"[tiab] OR "DNAm age"[tiab] OR "epigenetic age"[tiab] OR "methylation clock"[tiab] OR "methylation clocks"[tiab]) AND (trial[tiab] OR randomized[tiab] OR randomised[tiab] OR intervention[tiab] OR interventional[tiab] OR "pre-post"[tiab] OR "before and after"[tiab]) AND ("2010/01/01"[PDAT] : "2025/12/31"[PDAT]) AND (english[la] OR spanish[la]) AND humans[mh]	2010–2025; EN/ES; Humans; Article	145	145	See PRISMA
I2	PubMed (animals)	22/11/2025	("epigenetic clock"[tiab] OR "epigenetic clocks"[tiab] OR"DNA methylation age"[tiab] OR "DNAm age"[tiab] OR"epigenetic age"[tiab] OR "methylation clock"[tiab] OR"methylation clocks"[tiab])AND(trial[tiab] OR	2010–2025; EN/ES; Article; NOT humans	5	5	See PRISMA

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			randomized[tiab] OR randomised[tiab] ORintervention[tiab] OR treatment[tiab] OR"pre-post"[tiab] OR "before and after"[tiab])AND(Mice[MeSH Terms] OR mouse[tiab] OR murine[tiab] ORrat[tiab] OR rats[tiab] OR mammal*[tiab])AND("2010/01/01"[PDAT] : "2025/12/31"[PDAT])AND(english[la] OR spanish[la])NOT humans[mh]				
I3	Scopus	21/11/2025	TITLE-ABS-KEY(("epigenetic clock" OR "epigenetic clocks" OR "DNA methylation age" OR "DNAm age" OR "epigenetic age" OR "methylation clock" OR "methylation clocks") AND (trial OR randomized OR randomised OR intervention OR interventional OR "pre-post" OR "before and after"))	2010–2025; EN/ES; Article	507	507	See PRISMA
I4	Cochrane CENTRAL	21/11/2025	("epigenetic clock":ti,ab,kw OR "epigenetic clocks":ti,ab,kw OR "DNA methylation age":ti,ab,kw OR "DNAm age":ti,ab,kw OR "epigenetic age":ti,ab,kw OR "methylation clock":ti,ab,kw OR "methylation clocks":ti,ab,kw) AND (trial:ti,ab,kw OR randomized:ti,ab,kw OR randomised:ti,ab,kw OR intervention:ti,ab,kw OR "pre-post":ti,ab,kw OR "before and after":ti,ab,kw)	2010–2025; EN/ES	5	5	See PRISMA

Study Selection

Records were imported into Mendeley for de-duplication and management. Screening was performed in two phases (title/abstract, then full text). Final inclusion was determined by eligibility

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regarding population, design, and epigenetic outcomes. Selection is documented using a PRISMA flow diagram.

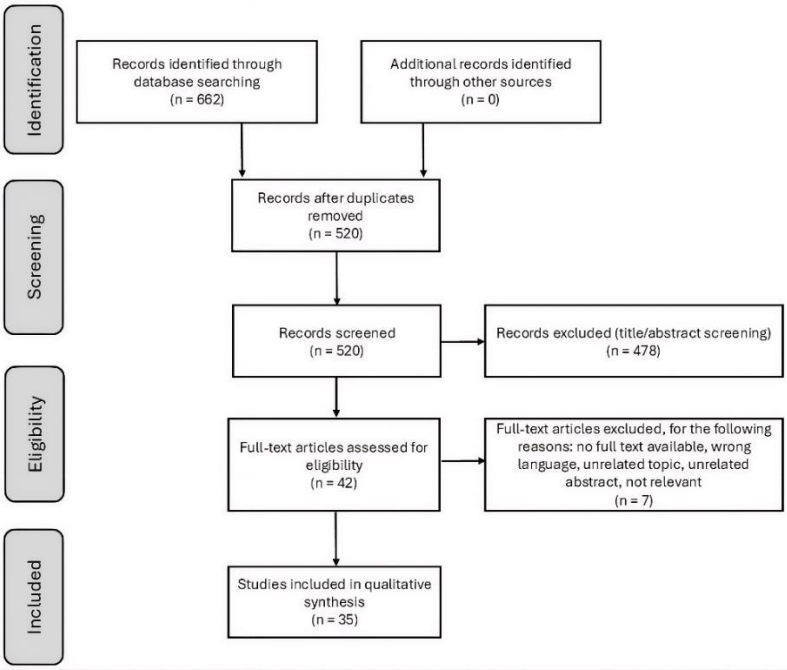

Figure 1. PRISMA flow diagram.

Screening, data extraction, and consistency checks were performed by the author.

Data Extraction

For included studies, data were extracted into a standardized spreadsheet capturing: ID, first author, year, title, country, species, design, sample size (total and per group), population description, intervention family and description, dose/schedule, comparator type and description, clock(s), tissue/matrix, timepoints, effects on DNAm age, effects on pace of aging, safety/adverse events, and overall risk of bias (RoB).

Due to space constraints, the full study-level extraction table (including study characteristics and overall RoB) is provided in Appendix B.

Risk of bias (RoB) Assessment

Study-level robustness was contextualized using a study-level risk-of-bias (RoB) field recorded in the extraction matrix, with a concise justification. In randomized trials, the overall RoB judgment was informed by an abbreviated RoB 2 approach, used for pragmatic study-level contextualization rather than formal domain-level adjudication. In non-randomized and/or preclinical studies, the RoB field captured major qualitative biases (e.g., confounding, selection, measurement), with supporting rationale recorded in the extraction matrix and reported in Appendix B.

Synthesis

Evidence was synthesized narratively and structured by intervention family: lifestyle, exogenous compounds, blood/circulation-derived interventions, and cellular reprogramming. Due to heterogeneity in interventions, populations, tissues, clocks, durations, and comparator structures (parallel vs pre–post), no meta-analysis and no formal publication-bias assessment were performed. Where studies reported multiple clocks and/or tissues, results were summarized per clock/tissue as reported, interpreting multiplicity cautiously and prioritizing pattern consistency (direction) over isolated point estimates.

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Results

Overview of Included Studies

Thirty-five longitudinal intervention studies in mammals were included, spanning diverse epigenetic clocks and biological matrices. Studies comprised predominantly human adult trials (including RCTs and single-arm pre–post designs), complemented by preclinical longitudinal studies in mammals (mouse, rat, and non-human primate). A wide range of clocks was used, including Horvath, Hannum, PhenoAge, GrimAge/GrimAge2, DunedinPACE, and species- or tissue-specific clocks in animal studies.[14-48]

Lifestyle Interventions

Thirteen studies modified lifestyle through diet, physical activity, weight management, and/or stress-related programs; all were human studies meeting inclusion criteria.[14-26] Across this category, follow-up ranged from ~8 weeks to 24 months (including structured 12–18-month programs), with sample sizes spanning from small pilot cohorts to >250 participants; for example, MACRO randomized n=148 (DNAm baseline n=144) and CALERIE randomized n=220 (DNAm n=197). Overall, lifestyle interventions tended to yield modest DNAm-clock changes, with more coherent signals under sustained exposures and plausible physiological shifts.

Dietary Interventions

Diet-focused interventions included sulfur amino-acid restriction, nut-based supplementation, dietary pattern comparisons, Mediterranean-style diets, very-low-calorie ketogenic diets, and chronic calorie restriction, with follow-up spanning weeks to two years.[14-20] In the STAY double-blind SAAR trial (NCT04701346; 8 weeks; n=59 randomized, SAAR n=31 vs control n=28), Hernández-Arciga et al. reported no significant changes in epigenetic clocks in blood (subset analyzed for methylation). Consistent with a null effect, estimated changes in epigenetic age measures were not distinguishable from zero and there were no significant between-group differences across the clocks evaluated after the 8-week intervention.[14] A 14-week mixed nut supplementation trial using a sperm-specific epigenetic clock reported no change in "germline age" despite differential methylation signals.[15] Trials comparing dietary patterns (e.g., low-carbohydrate vs low-fat) showed small changes with limited between-group differentiation across clocks, including only slight shifts in DunedinPACE (overall ~1.00 to 0.99 at 12 months; ~0.01 units) and modest/discordant patterns in other clocks (including slight increases in PCPhenoAge and PCGrimAge in the low-carbohydrate arm vs low-fat in MACRO).[16] In a small longitudinal obesity cohort undergoing a very-low-calorie ketogenic diet (VLCKD) (n=10), epigenetic age deceleration was observed both during nutritional ketosis (~30 days; Horvath −3.3, Hannum −6.3, Levine −8.8 years) and at 180 days (Horvath −1.1, Hannum −7.4, Levine −8.2 years), with a mean slowing of approximately −6.1 years during ketosis and −6.2 years at study end ($p<0.0001$). Greater slowing was associated with BMI reduction, higher β-hydroxybutyrate levels ($r\approx-0.67$ to -0.75; $p\leq0.001$), and broader metabolic improvements.[17] An individualized Mediterranean diet program did not show significant changes in Horvath age acceleration across the full cohort, with only a small subgroup signal that did not generalize across strata, with the epigenetic analysis conducted in n=120 intervention participants and significance (after BH correction) restricted to a subgroup of Polish women for AgeAccel/IEAA.[18] A 4-week dietary intervention in 32 adults with metabolic syndrome using daily tree nuts + extra-virgin olive oil showed no significant change in epigenetic aging measures despite elevated baseline aging rates, with ΔDunedinPACE = −0.002 ± 0.070 ($p=0.86$) and ΔAgeAccelGrim = −0.04 ± 1.34 ($p=0.89$).[19]

By contrast, sustained calorie restriction produced clearer effects on pace-of-aging outcomes. In CALERIE (25% target restriction; achieved ~12% on average over two years), DNAm age clocks did not differ between groups, but DunedinPACE decreased moderately in the calorie restriction arm, with larger effects among participants achieving higher restriction levels, with effect sizes of

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approximately d=−0.29 at 12 months (p=0.0004) and d=−0.25 at 24 months (p=0.008) overall, and up to d=−0.43 at 12 months ($p=1.4\times10^{-5}$) and d=−0.40 at 24 months (p=0.0002) among participants achieving ~20% calorie restriction.[20] These data suggest that clocks capturing pace/functional decline may respond more robustly to sustained metabolic shifts than clocks optimized for chronological age.

Physical Activity Interventions

Pure exercise-only evidence was more limited. A pilot home-based exercise program (GO-EXCAP) in older adults with myeloid neoplasms reported moderate but non-significant decreases in GrimAge and PhenoAge, with median [IQR] changes of approximately −1.4 years for both clocks (p=0.55 and p=0.10, respectively), with no consistent change in DunedinPACE or first-generation clocks (DunedinPACE median [IQR] −0.1 [0.2]; p=0.47).[21] In the Florence DAMA trial physical activity did not significantly affect GrimAge ($\beta = 0.09$; $p = 0.73$) but did significantly reduce epigenetic mutation load (EML) ($\beta = -2.06$; 95% CI −2.84 to −1.28; $p < 0.001$). [22]

Multicomponent Programs

Four multicomponent interventions combined diet, exercise, structured weight management, and/or stress reduction; none reported adverse events.[23-26] An 8-week multicomponent program (plant-centered diet designed to support methylation, targeted supplementation, probiotics, and structured exercise) reported a significant saliva Horvath age reduction compared with controls, with a between-group difference of approximately −3.23 years (p=0.018; treatment n=18 vs control n=20).[23] DIRECT-PLUS (18 months; dietary counseling including a polyphenol-rich variant with workplace physical activity support) evaluated multiple blood clocks and reported modest differences associated with adherence, including modest Li DNAmAge increases of ~0.8–1.1 years across groups over follow-up, with no clear between-diet pattern for DunedinPACE (which decreased similarly across groups without significant between-pattern differences).[24] Other structured weight-management programs showed small, often non-significant changes across clocks, including ~0.5–1.1-unit reductions in Horvath and Hannum clocks over 12 weeks that did not reach statistical significance.[25] A stress-response relaxation intervention reported an average epigenetic age decrease of ~1.5 years, with stronger signal in healthy participants than in post-myocardial-infarction patients, but effects were borderline and heterogeneous, with −4.67 ± 4.40 years in healthy participants (p=0.053) versus −0.14 ± 1.55 years in post-MI patients (p=0.428).[26]

Exogenous Compounds (Humans and Preclinical)

Fifteen studies evaluated exogenous compounds: pharmacological agents, vitamins, nucleotide supplementation, polyphenols/extracts, NAD^+ modulators, and multinutrient formulations.[27-41] Thirteen were human studies and two were in mammalian models (marmosets and mice). Overall, heterogeneity was high and coherence across clocks and matrices was limited.

Pharmacological Interventions

In a factorial trial of postmenopausal women with overweight and prior breast cancer, metformin (up to ~850 mg twice daily), a telephone-based weight-loss program, and their combination were compared with placebo/standard care over six months with multiple blood clocks assessed. Epigenetic findings were globally null for metformin versus placebo across clocks, with adjusted ITT differences in age acceleration ranging from −0.91 to +0.82 years across clocks (all $p \geq 0.18$), including EAA PhenoAge +0.82 years (95% CI −1.16 to 2.80; p=0.41) and EAA GrimAge −0.91 years (95% CI −2.24 to 0.41; p=0.18), and the weight-loss arm showed small, inconsistent signals interpreted as statistical noise, including a nominal increase in EAA PhenoAge of +2.02 years (95% CI 0.02 to 4.03; p=0.05, adjusted ITT).[27]

In an RCT in mild-to-moderate Alzheimer's disease, bezisterim (NE3107/HE3286; 20 mg twice daily for 30 weeks) was associated with multi-year epigenetic age reductions versus placebo in a

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small per-protocol methylation subset across several blood clocks (including SkinBloodAge, Hannum, and InflammAge), with trends in GrimAge and PhenoAge, with point estimates of approximately −3.68 years (SkinBloodAge; p=0.017), −5.00 years (Hannum; p=0.006), −4.77 years (InflammAge; p=0.022), and trend-level reductions of −3.71 years (PhenoAge) and −1.92 years (GrimAge; p≈0.06–0.08).[28] Safety reporting indicated common treatment-emergent adverse events with no excess discontinuations in the active arm versus placebo, with any TEAE in 72.7% vs 62.5%, treatment-related AEs in 12.5% vs 18.2%, discontinuations due to AEs in 0% vs 9.1%, and SAEs in 4.2% vs 9.1% (bezisterim vs placebo, per-protocol population).[28]

In preclinical evidence, rapamycin showed discordant results across species and tissues: in common marmosets, chronic rapamycin did not change blood epigenetic age, with a small non-significant treatment coefficient of −0.18 years (p=0.686);[29] in mice, dietary rapamycin, calorie restriction, and Ames dwarfism markedly slowed hepatic epigenetic aging (based on WGBS, n=4 per group), with stronger effects for calorie restriction and Ames dwarfism than rapamycin, with reductions of ~6.0 months for rapamycin ($p<0.05$), ~9.4 months for calorie restriction ($p<10^{-4}$), and ~10.1 months for Ames dwarfism ($p<0.01$).[30]

Vitamins and Omega-3

In DO-HEALTH (2×2×2 factorial; older European adults), daily vitamin D_3 and omega-3 supplementation, with or without a simple home strength program, was associated with small favorable shifts in blood PC-PhenoAge over three years, with global effect sizes of ~0.16–0.32 units (≈2.9–3.8 months equivalent), with the most consistent omega-3 effects observed in PhenoAge, GrimAge2, and DunedinPACE.[31] A longitudinal cohort of vitamin-D–deficient older adults reported lower epigenetic age in those self-reporting supplementation versus matched quasi-controls using a 7-CpG clock and Horvath, by approximately 2.6 years (7-CpG clock; p=0.011) and 1.3 years (Horvath; p=0.042), respectively, with no differences in Hannum, PhenoAge, or GrimAge.[32] Two studies explored B vitamins: folic acid plus B12 over two years did not significantly change blood Horvath age overall, with a mean change of approximately −0.765 ± 1.435 years (p=0.60; n=44), though a genotype-specific signal was reported;[33] another trial adding folate/B6/B12 to vitamin D_3 and calcium produced divergent CpG-level changes in a reduced CpG clock, with inconclusive net effects on global epigenetic age, including ΔASPA 1.40 ± 4.02 vs −0.96 ± 5.12 (p=0.046), ΔPDE4C 1.95 ± 3.57 vs 0.22 ± 3.57 (adjusted p=0.062), and an adjusted OR of 5.26 (95% CI 1.51–18.28) for "accelerated aging" in the B-vitamin group.[34]

Multinutrient Formulations

A 12-week open trial of a combined supplement including vitamins, polyphenols, and omega-3 in middle-aged and older adults showed no significant overall changes across major clocks, with subgroup signals restricted to participants with higher baseline acceleration and to saliva InflammAge in a defined subgroup, including an approximately 2-year reduction in a Horvath-accelerated subgroup (p≈0.069), and in the saliva InflammAge-accelerated subgroup (n=29), reductions of ≈3.31 years in epigenetic age (−4.055%; p=0.015) and ≈3.47 years in age acceleration (−46.77%; p=0.0058).[35] An open-label 12-month "Cel System" program combining multicomponent capsules with minimal walking and meditation reported a reduction in PC Horvath age acceleration at 12 months from 0.60 to −0.15 years (Δ≈−0.75; p=0.048), with a larger effect at 6 months (−0.36; $p\approx6.1\times10^{-4}$) and reductions in a damage-centered clock (DamAge) at earlier timepoints, from 2.46 to approximately −0.7 years at 3–6 months (p≈0.003–0.0014), with partial rebound to about −0.12 years at 12 months (p=0.12), while DunedinPACE increased over 12 months, from 0.94 to 0.99 (~5% acceleration; $p\approx7.4\times10^{-5}$).[36] A separate open study of Ca-AKG plus vitamin A or D using a proprietary 9-CpG saliva clock reported a large mean epigenetic age reduction over ~7 months, with a mean reduction of 7.96 years (n=42; $p=6.538\times10^{-12}$), and −7.69 years in a "stable lifestyle" subgroup (n=13; $p=7.263\times10^{-5}$), without adverse events reported.[37]

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Polyphenols/Extracts and Nucleotide Supplementation

A 90-day open pilot using HBT Rejuvenate (Himalayan tartary buckwheat–based formulation) reported no significant changes in blood OMICmAge, PCPhenoAge, PCGrimAge, or DunedinPACE overall, though subgroup analyses by baseline level showed changes in age-acceleration metrics without global effects, including decreases in PCPhenoAge EAA in the +1 SD baseline subgroup ($p=0.031$) and increases in PCGrimAge EAA and OMICmAge EAA in −1 SD baseline subgroups (both $p=0.031$).[38] A 12-week randomized trial of Monarda didyma L. extract (100 mg/day) reported stability of epigenetic age in the intervention group while placebo increased significantly, resulting in a significant between-group difference at week 12 using a 5-CpG clock, with within-group $p=0.4522$ for intervention (stable) versus $p<0.0001$ for placebo (increase), and a post-intervention between-group difference of $p=0.0162$; no adverse events were reported.[39] In TALENTs, 5′ nucleotide supplementation (AMP, CMP, GMP, UMP; 1.2 g/day for 19 weeks) in older adults reduced a composite epigenetic age metric (median across PC Horvath, Hannum, GrimAge, and PhenoAge) by ~3.1 years versus placebo at end-of-follow-up, with $\beta \approx -3.08$ years (95% CI −5.07 to −1.10; $p \approx 0.0023$), with no serious adverse events or clinically relevant safety changes.[40]

NAD^+ Modulators

In a 10-week double-blind RCT in older adults with mild cognitive impairment, nicotinamide riboside (up to 1 g/day) did not produce significant within-group changes or differences versus placebo across IEAA, EEAA, PhenoAge, or GrimAge measured in PBMCs; exploratory trends were small and inconsistent.[41] Adverse events were recorded in both arms, with no serious adverse events reported; 18 adverse events occurred in the NR arm and 21 in placebo (7/10 participants in each arm), including one stroke in placebo and one case of severe nausea in the active arm that improved after dose reduction.[41]

Blood/Circulation-Derived Interventions (Humans and Rodents)

Six studies evaluated blood- or circulation-derived interventions: three in humans (blood products and plasma exchange-related procedures) and three in rodent models (young plasma, plasma fractions/exosome-rich preparations, and heterochronic parabiosis).[42-47]

Human Studies

A single-site sham-controlled trial assessed therapeutic plasma exchange (TPE) with albumin, with one arm including intravenous immunoglobulin (IVIG), under different schedules over 3–6 months in a 42-participant trial, quantifying 36 blood methylation clocks, with biweekly (TPE±IVIG) and monthly TPE regimens. Two discontinuations occurred due to adverse events, one linked to IVIG, and a mild albumin allergic reaction occurred in 0.42% of procedures (1/240 procedures).[42] At the end of the intervention period (second timepoint, before session 4), the maximum epigenetic age decrease versus sham was ~2.6 years in the TPE+IVIG arm and ~1.3 years in a monthly TPE arm, specifically 2.61 years ($FDR=6.22\times10^{-5}$) and 1.32 years ($FDR=2.42\times10^{-2}$), respectively, with consistent direction across multiple clocks ($FDR<0.05$), including 10 clocks differing vs sham in TPE+IVIG and 5 clocks in monthly TPE, and particularly large decreases (~7–10 years) in immune- and inflammation-centered clocks (≈9.7 years for “Immune” and ≈7.1 years for “Inflammation”).[42] Notably, at a later follow-up after sessions stopped, the differences disappeared versus sham, consistent with signal loss after discontinuation (no significant differences vs sham at the later post-intervention assessment).[42]

In contrast, another study examined repeated plasmapheresis in human donors under two different frequencies without a true no-treatment arm (a delayed-start crossover-like control), in which one group did not undergo the first 4 plasmapheresis procedures and functioned as a crossover-like control. Over 18 weeks (up to 8 procedures), with 570–830 mL removed per session, mixed models indicated significant increases in multiple GrimAge family clocks (~+0.16–0.26 years

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per session) and Hannum-type clocks (~+0.13–0.17 years per session), including GrimAge +0.26±0.05 ($p=5\times10^{-7}$), GrimAge2 +0.22±0.05 (p=0.0002), GrimAge2_Tuned +0.16±0.03 ($p=1.26\times10^{-5}$), GrimAge2_Calibrated +0.22±0.05 (p=0.0002), Hannum +0.17±0.04 (p=0.0002), and RobustHannum +0.13±0.03 ($p=2.42\times10^{-5}$), alongside an increase in DunedinPACE of ~0.003±0.001 units per session (p=0.0058), corresponding to ≈2.4% cumulative acceleration after 8 sessions. No adverse events were reported.[43]

Finally, an open study administered a human umbilical cord plasma concentrate (secretome enriched in extracellular vesicles and proteins) via weekly intramuscular injections for 10 weeks (1 mL weekly; n=18 adults). The primary signal was a reduction in GrimAge acceleration (~0.82 years), from approximately +0.04 years to −0.78 years (paired p≈0.009), with decreases in methylation-based protein surrogates (Cystatin C and GDF-15) (age-adjusted $p=2.4\times10^{-2}$ and $p=2.4\times10^{-3}$, respectively), while classic clocks (Horvath, Hannum, SkinBloodAge, PhenoAge, DNAmTL, DNAmGrimAge) did not change significantly. Local mild reactions occurred in two participants after the first injection, with no other adverse events reported.[44]

Preclinical Studies

Across three rodent studies, reported epigenetic age reductions were large—up to ~ 77.6%—across multiple organs in some designs. One study administered an exosome-rich plasma fraction (E5) from young pigs to old Sprague-Dawley rats and measured rat-specific epigenetic clocks in multiple organs, using 109-week-old (~25-month) male rats (n=6/group) and E5 derived from 6–7-month-old pigs, delivered as two series of four intravenous injections separated by 95 days (total study duration 155 days), reporting large reductions (e.g., liver 77.6%, blood 68.2%, heart 56.5%, hypothalamus 29.6%), with a mean ~67% rejuvenation across four primary tissues. A replication experiment (E5 vs saline) confirmed significant blood rejuvenation on the final blood clock, with a weaker signal on the pan-tissue clock (p=0.054); after excluding one outlier control, p=0.014 in females and p=0.053 in males, with no evident abnormal physical/behavioural signs or histological alterations.[45] Another study administered young rat plasma to very old female rats until death, via 1 mL intraperitoneal injections every 2 weeks (plasma n=9 vs control n=8) starting at ~25.6 months, reporting improved appearance parameters, increased median lifespan by 2.2 months, and lower blood epigenetic age; the clearest signal emerged in an age-band analysis late in life (27–31.5 months) showing ~3–4 months lower epigenetic age versus controls ($p<0.05$).[46]

A heterochronic parabiosis study in old mice exposed to young circulation for three months (followed by surgical separation) assessed blood and liver using murine epigenetic clocks and multi-omic platforms in C57BL/6J mice (old ~20 months), compared with old–old isochronic controls. After separation, old mice previously exposed to young circulation showed ~16–32% lower blood epigenetic age than old–old controls, with liver reductions of ~17–27% (array-based) and sustained rejuvenation (~11–26%) after separation; during the parabiosis phase, liver RRBS-based reductions ranged ~5–26%; short-term parabiosis (5 weeks) produced much smaller and often non-significant changes (~0–11%), indicating strong dependence on exposure duration.[47]

Cellular Reprogramming (Preclinical)

No human interventions met criteria for cellular reprogramming. Evidence was limited to a single study in aged female Sprague-Dawley rats assessing OSKM factor expression in hippocampus.[48] Old rats received a stereotaxic bilateral injection of a high-capacity adenovector (Tet-Off cassette) expressing OSKM plus GFP, with 39 days of expression before sacrifice, compared with old GFP-only controls and young intact rats (young intact: 3.5 months, N=12; old GFP controls: 25.3 months, N=16; old OSKM-GFP: 25.3 months, N=17). No pathological alterations were observed in hippocampus or other brain regions within the expression window.[48] Three hippocampus-based clocks were evaluated (rat brain clock, human–rat relative age clock, and a mouse brain clock adapted to MammalMethylChip40), all measured in hippocampal tissue at the end of the 39-day expression window, with the main old-vs-old comparison based on n=6 controls vs n=8 OSKM-treated rats.

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Compared with old controls, OSKM-treated old rats showed slightly lower epigenetic age across clocks, with near-significant two-sided p-values (0.064, 0.076, 0.088) and $p<0.05$ in one-sided contrasts; no pace-of-aging metrics analogous to DunedinPACE were assessed.[48]

Discussion

Across intervention families, the evidence supports measurable plasticity of DNAm-based biomarkers in response to interventions, but with strong dependence on intervention type, clock construct, tissue/matrix, duration, and methodological robustness. In many settings, the same intervention can be associated with improvements in certain clocks and no change—or discordant change—in others, underscoring the need to interpret effects relative to what each clock measures.[8,9,11-13]

Lifestyle Interventions: Modest Effects, More Coherent Under Sustained Exposure

Across 13 human studies, lifestyle interventions varied widely in design and clocks, yet the overall pattern was relatively stable: effects tended to be small-to-moderate, and the most consistent signals emerged when exposures were sustained (months to years) and accompanied by plausible physiological shifts, such as prolonged calorie restriction.[20] By contrast, short interventions and dietary "composition" comparisons often produced small changes with limited between-group differentiation, consistent with the interpretation that clock signals respond more robustly to chronic systemic state changes (energy balance, adiposity, inflammation, metabolic signalling) than to isolated dietary modifications without sustained systemic impact.

Exogenous Compounds: High Heterogeneity and Vulnerability to Non-Reproducible Signals

For exogenous compounds (pharmacological agents and nutraceuticals), heterogeneity was greater and global coherence weaker. The contrast between a robust trial with globally null epigenetic findings (metformin)[27] and favorable signals in a small per-protocol sub-study (bezisterim)[28] illustrates that mechanistic plausibility does not guarantee detectable effects on epigenetic clocks—particularly when analytic sample sizes are small or when multiple clocks are assessed without a clearly pre-specified primary endpoint. For nutraceuticals and complex formulations, a substantial portion of evidence derives from open-label and/or uncontrolled studies, increasing vulnerability to selection bias, regression to the mean, concurrent lifestyle changes, and expectation effects, and complicating causal attribution.[37] The use of proprietary clocks or reduced CpG panels adds another interpretive layer: large decreases in a specific clock may reflect real shifts in a particular signature, but may also be contingent on clock training, matrix (saliva vs blood), and technical stability.

In animals, the contrast between null blood findings in primates (rapamycin)[29] and clearer effects in solid tissues in mice (liver)[30] reinforces tissue dependence. These observations support the notion that intervention studies should justify tissue and clock selection explicitly; measuring blood for all interventions is not methodologically equivalent, especially when expected mechanisms are tissue-specific or when signals may be diluted by mixed-cell composition.

Blood/Circulation-Derived Interventions: Striking Effects, Yet Transient and Interpretively Ambiguous

Blood/circulation interventions produced some of the largest reported effect sizes, but also carry substantial potential for alternative interpretations. In humans, sham-controlled therapeutic plasma exchange provides methodologically informative evidence that altering the plasma environment can be reflected in epigenetic clocks, particularly those sensitive to immune/inflammation signatures, with a maximum decrease of ~2.6 years in the TPE+IVIG arm (and ~1.3 years in a monthly TPE arm) at the end of the intervention period.[42] However, loss of signal after sessions stop suggests that at least part of the observed change may be transient or maintenance-dependent, shifting interpretation toward "systemic state modulation" rather than stable reversal of underlying biology.[42]

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In rodents, large percentage reductions across organs after plasma fractions/exosome-rich preparations or heterochronic parabiosis broaden the plausibility of a modifiable systemic component, with reported reductions up to ~77.6% in some tissues/designs.[45] Nevertheless, due to magnitude, these results warrant special caution for translation: small sample sizes, dependence on exposure duration, potential influence of tissue composition, and the need for independent replication. Even if effects are real in rodents, translation to humans requires clarifying how much observed change reflects "biological age" versus "tissue state" and cellular composition.

Partial Reprogramming: Early, Conceptual Evidence with Small Effects

Partial reprogramming evidence remains incipient and limited to a single rat study with focal hippocampal assessment.[48] Consistent trends toward younger epigenetic age with near-significant p-values (two-sided p=0.064–0.088 across three clocks) are compatible with a small-to-moderate effect under conservative expression conditions (expected given safety constraints) and/or insufficient power to robustly detect epigenetic changes in brain tissue. At this stage, the principal value of these data is conceptual: they support in vivo feasibility of directly intervening on epigenetic states, but do not yet support firm inferences about durability, therapeutic window, or generalizability.

Limitations

Three limitations cut across categories and should be incorporated explicitly in interpretation.

1) Multiplicity of clocks and endpoints: Many studies report multiple clocks and sub-analyses, increasing the risk of isolated, non-reproducible signals when correction or pre-specification is absent.

2) Matrix and cellular composition: Whole blood, PBMCs, and saliva are not equivalent. Some clocks are highly sensitive to immune/inflammatory and cell-composition shifts, which may yield apparent "rejuvenation" that partially reflects transient state changes.

3) Design and causal attribution: The most striking findings in several nutraceutical and systemic interventions frequently come from open-label or uncontrolled designs, limiting causal attribution and tending to inflate effect estimates.

Additionally, evidence heterogeneity (populations, interventions, tissues, clocks, and parallel vs pre–post schemes) precluded meta-analysis and requires cautious interpretation of effect magnitude. Search scope was restricted by time period, language, and databases consulted, and screening/extraction were conducted by a single author, despite documented traceability.

Conclusions

DNAm-based epigenetic biomarkers are modifiable by interventions in mammals, but observed effects are heterogeneous and depend on the intervention, the clock construct (age versus pace/risk signatures), the biological matrix and tissue, and study design. A single notion of "epigenetic rejuvenation" is not supported; rather, intervention effects appear domain-specific and should be interpreted in relation to what each clock measures.

In humans, lifestyle interventions generally show modest effects, with more consistent signals when stimuli are sustained and accompanied by physiologically meaningful changes. For exogenous compounds, evidence is mixed: robust null findings coexist with favorable signals in other contexts, often conditioned by clock heterogeneity, small analytic samples, or open-label designs. For blood/circulation-derived interventions, human studies suggest that manipulating the circulatory milieu can be reflected in immune/inflammation-sensitive clocks, but direction and persistence are not uniform; in animal models, larger-magnitude effects are reported with substantial translational uncertainty. Partial reprogramming evidence remains preclinical and preliminary and is best considered conceptual rather than confirmatory. Overall, results support the premise that intervening on biological determinants of aging can modify epigenetic readouts, while underscoring

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the need for stronger standardization to distinguish transient "state modulation" from sustained changes compatible with slowing biological aging.

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Use of generative AI tools: Generative AI tools were used exclusively for auxiliary support (preliminary spelling/grammar checks, suggestions for reference organization/formatting, synonym exploration and sentence rephrasing for clarity, and initial drafts of some schematics based on author-defined concepts). No AI tools were used for study selection, data extraction, critical analysis, or conclusions. All AI-assisted outputs were manually validated.

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Appendix A. Full Search Strategy (Search Terms and Strings by Database)

A1. Mechanistic Evidence Search Strategy (PubMed)

ID	Database	Date	Query	Filters	Results	Exported	Selected
M0	PubMed	17/11/2025	(“hallmarks of aging”[tiab] OR “hallmarks of ageing”[tiab]) AND (update[tiab] OR review[pt] OR framework[tiab]) AND (2010:2025[dp])	2010–2025[dp]; review[pt]	475	2	36599349; 23746838
M1	PubMed	17/11/2025	(epigenetic*[tiab] AND (aging[tiab] OR ageing[tiab])) AND (review[pt] OR mechanisms[tiab] OR framework[tiab]) AND (2010:2025[dp])	2010–2025[dp]; review[pt]	3831	3	36522308; 29643443; 36336680
M2	PubMed	17/11/2025	(“DNA methylation”[tiab] OR “DNA Methylation”[Mesh]) AND (dynamics[tiab] OR remodeling[tiab] OR turnover[tiab] OR demethylation[tiab] OR “epigenetic drift”[tiab]) AND (aging[tiab] OR ageing[tiab] OR “Aging”[Mesh]) AND (review[pt] OR “Review”[Publication Type] OR mechanisms[tiab]) AND (2010:2025[dp]) NOT (atherosclero*[tiab] OR cardiomyocyte*[tiab] OR cancer*[tiab] OR tumor*[tiab] OR Huntington*[tiab] OR plaque[tiab])	2010–2025[dp]; review/Review; NOT (cardio/cáncer/etc.)	180	3	25913071; 32356238; 29268958
M3	PubMed	17/11/2025	(chromatin[tiab] OR heterochromatin[tiab] OR “H3K9me3”[tiab] OR “H3K27me3”[tiab] OR sirtuin*[tiab] OR polycomb[tiab] OR histone*[tiab]) AND (aging[tiab] OR ageing[tiab]) AND (review[pt] OR mechanisms[tiab]) AND (2010:2025[dp])	2010–2025[dp]; review[pt]	2976	2	27518561; 27482540
M4	PubMed	17/11/2025	(“DNA methylation age”[tiab] OR “epigenetic clock”[tiab] OR “epigenetic age”[tiab] OR PhenoAge[tiab] OR GrimAge[tiab] OR DunedinPACE[tiab]) AND (human*[tiab] OR tissue*[tiab]) AND (2010:2025[dp])	2010–2025[dp]; human*/tissue*	718	4	24138928; 29676998; 36516495; 35029144

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A2. Intervention Search Strategy (PubMed, Scopus, and CENTRAL)

ID	Database	Date	Query	Filters	Results	Exported	Selected
I1	PubMed (humans)	21/11/2025	("epigenetic clock"[tiab] OR "epigenetic clocks"[tiab] OR "DNA methylation age"[tiab] OR "DNAm age"[tiab] OR "epigenetic age"[tiab] OR "methylation clock"[tiab] OR "methylation clocks"[tiab]) AND (trial[tiab] OR randomized[tiab] OR randomised[tiab] OR intervention[tiab] OR interventional[tiab] OR "pre-post"[tiab] OR "before and after"[tiab]) AND ("2010/01/01"[PDAT] : "2025/12/31"[PDAT]) AND (english[la] OR spanish[la]) AND humans[mh]	2010–2025; EN/ES; Humans; Article	145	145	See PRISMA
I2	PubMed (animals)	22/11/2025	("epigenetic clock"[tiab] OR "epigenetic clocks"[tiab] OR"DNA methylation age"[tiab] OR "DNAm age"[tiab] OR"epigenetic age"[tiab] OR "methylation clock"[tiab] OR"methylation clocks"[tiab])AND(trial[tiab] OR randomized[tiab] OR randomised[tiab] ORintervention[tiab] OR treatment[tiab] OR"pre-post"[tiab] OR "before and after"[tiab])AND(Mice[MeSH Terms] OR mouse[tiab] OR murine[tiab] ORrat[tiab] OR rats[tiab] OR mammal*[tiab])AND("2010/01/01"[PDAT] : "2025/12/31"[PDAT])AND(english[la] OR spanish[la])NOT humans[mh]	2010–2025; EN/ES; Article; NOT humans	5	5	See PRISMA
I3	Scopus	21/11/2025	TITLE-ABS-KEY(("epigenetic clock" OR "epigenetic clocks" OR "DNA methylation age" OR "DNAm age" OR "epigenetic age" OR "methylation clock" OR "methylation clocks") AND (trial OR randomized OR randomised OR intervention OR interventional OR "pre-post" OR "before and after"))	2010–2025; EN/ES; Article	507	507	See PRISMA
I4	Cochrane CENTRAL	21/11/2025	("epigenetic clock":ti,ab,kw OR "epigenetic clocks":ti,ab,kw OR "DNA methylation age":ti,ab,kw OR "DNAm age":ti,ab,kw OR "epigenetic age":ti,ab,kw OR "methylation clock":ti,ab,kw OR "methylation clocks":ti,ab,kw) AND (trial:ti,ab,kw OR randomized:ti,ab,kw OR	2010–2025; EN/ES	5	5	See PRISMA

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			randomised:ti,ab,kw OR intervention:ti,ab,kw OR "pre-post":ti,ab,kw OR "before and after":ti,ab,kw)					

Appendix B. Extraction table

Table B1. Extraction matrix for studies included in the qualitative synthesis (human and preclinical), including study characteristics, intervention/comparator details, epigenetic clock outcomes, safety/adverse events, and study-level risk-of-bias (RoB) judgments.

Paper										Intervention			Comparator		Epigenetic_outcome					Secondary_outcome	Risk_of_bias
ID	First_author	Year	Title	Country	Specie	Design	N_total	N_per_group	Population_description	Intervention_family	Intervention_description	Dose_schedule	Comparator_type	Comparator_description	Clock_name	Tissue	Timepoints	Effect_on_DNAmAge	Effect_on_pace_of_aging	Safety_AEs	RoB_overall
7	Fitzgerald, KaraN	2021	Potential reversal of epigenetic age using a diet and lifestyle intervention: a	USA	Human	Randomized controlled parallel-group pilot trial; 8-week multimodal lifestyle	43 randomized (38 analysed for primary outcome)	Intervention 21 randomized (18 analysed); Control 22 randomized (20 analysed)	Community-dwelling men aged 50–72 years without recent or chronic disease (cardiovascular, diabetes, autoimmune, cancer,	Lifestyle	8-week plant-centered, methylation-supportive diet plus PhytoGanix® and UltraFlora® with structured exercise.	8-week intervention; diet emphasizing high intake of dark leafy greens, overnight 12-hour fast (~7pm–7am),	No-intervention / usual lifestyle control	Control group received no specific dietary, supplemental, exercise, sleep or stress-management interventi on;	Horvath DNAmAge	Saliva	Baseline and end of 8-week intervention (study visit at ~week 9)	Compared with controls, intervention participants were on average 3.23 years younger at end of study (between-group difference, p=0.018). Within the intervention	NR	NR (no explicit adverse event or safety data reported)	(RoB 2) Overall some concerns: randomization and concealment were adequate with similar baseline

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			pilot randomized clinical trial.			intervention vs no-intervention control			neurodegenerative, etc.), non-smokers, limited alcohol, recruited around Portland, Oregon.			reduced refined carbohydrates, avoidance of added sugar and most processed foods; supplements: PhytoGanix® 2 servings/day and UltraFlora® Intensive Care 2 capsules/day (L. plantarum 299v); lifestyle: ≥30 minutes		continued usual habits and any pre-existing low-dose supplements allowed by protoco				arm, DNAmAge decreased by mean 1.96 years vs baseline (p=0.066), while controls increased by 1.27 years (p=0.153). No net change in overall methylation level of the 353 Horvath clock CpGs; change reflected repositioning of methylation patterns toward a younger profile			s, and DNAmAge (Illumina EPIC) was analysed blinded despite unblinded participants/coaches, but attrition was moderate and slightly unbalanced (18/21 vs 20/22 analysed) with limited reasons

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											moderate-intensity exercise 5 days/week target ≥7 hours sleep per night.									reported, and the small pilot sample plus no detailed pre-published analysis plan keep the rating at some concerns.
9	Bischoff-Ferrari, Heike A	2025	Individual and additive effects of vitamin D, omega	Switzerland	Human	Post hoc analysis of randomized, double-blind, placebo-	777	Placebo 95; Vitamin D only 101; Omega-3 only 98; SHEP only 92;	Community-dwelling adults ≥70 years (mean age ~75.5, ~60% women), generally	Exogenous compound–based	Daily vitamin D3 and omega-3 supplementation with or without a simple home	Vitamin D3 2,000 IU/day; omega-3 1 g/day (330 mg EPA + 660 mg DHA from	Placebo	Vitamin D and omega-3 placebos identical in appearance, taste and weight;	PC-PhenoAge	Whole blood	Baseline and 3-year follow-up	Omega-3 vs no omega-3 reduced PhenoAge age-acceleration change (standardized d ≈ −0.16; 95% CI −0.30 to	NR	NR (no explicit adverse event or safety data reported)	RoB2 – overall low to some concerns. Parent DO-HEALTH trial

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			-3 and exercise on DNA methylation clocks of biological aging in older adults from the DO-HEALTH trial.			controlled 2×2×2 factorial RCT (DO-HEALTH) with 3-year vitamin D, omega-3 and home exercise interventions		Vitamin D + Omega-3 95; Vitamin D + SHEP 104; Omega-3 + SHEP 95; Vitamin D + Omega-3 + SHEP 97	healthy and active (no major cardiovascular events or cancer in prior 5 years, MMSE ≥24, independent mobility)		strength exercise program (SHEP)	marine algae) given as two identical gel capsules per day; SHEP strength-training 30 min three times per week for 3 years versus attention-control joint-flexibility exercises 30 min three times per week		attention-control flexibility exercise program 30 min three times weekly; factorial design comparing vitamin D vs placebo, omega-3 vs placebo and SHEP vs control exercise plus their combinations over 3 years			−0.02), corresponding to ~3 months slower biological aging over 3 years; vitamin D alone and SHEP alone showed no significant effects; combinations including omega-3 plus vitamin D and/or SHEP showed additive benefits on PhenoAge (d ~ −0.24 to −0.32)			was a large, multicenter, randomized, double-blind, placebo-controlled 2×2×2 factorial RCT with centralized randomization and matching placebos; exercise arms used an attention-

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																				control flexibility program, with blinding less feasible. DNAm assays were performed on stored blood with laboratory staff blinded to treatment allocation.

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11	Fiorito, Giovanni	2021	DNA methylation-based biomarkers of aging were slowed down in a two-year diet and physical activity intervention trial: the DAMA study.	Italy	Human	24-month randomized 2×2 factorial lifestyle RCT (diet, PA, diet+PA, control); DNAm outcomes = secondary analysis	219 paired samples (post-QC)	Diet 57 → 56; PA 56 → 56; Diet+PA 53 → 53; Control 58 → 54 (final pairs aggregated to N=219)	Healthy postmenopausal women (50–69 y) with high mammographic density (>50%), nonsmokers, attending Florence breast cancer screening program	Lifestyle	Plant-based, low-glycemic-load diet; structured physical activity program (~1 h/day moderate + 6–10 MET-h/week vigorous); combined arm = both; control = minimal lifestyle advice	24 ± 3 months; diet: counseling + 6 group sessions + 8 cooking classes; PA: supervised weekly 1-h session + home exercises + walks	Active minimal-intervention lifestyle advice	– Diet effect = diet-containing arms (diet + diet+PA) vs no-diet arms (PA + control) – PA effect = PA-containing arms (PA + diet+PA) vs no-PA arms (diet + control)	DNAmGrimAge, DNAmGrimAge Acceleration (DNAmGrimAA), Epigenetic Mutation Load (EML; stochastic epigenetic mutations), GrimAge components (PAI-1, Leptin, GDF-15, etc.)	Whole blood (buffy coat)	Baseline and 24 months	Diet slowed aging: – Within-group: diet −0.41 y (95% CI −0.79, −0.03) vs controls +0.25 y – DiD (diet vs control): β = −0.66 y (95% CI −1.15, −0.17; p=0.01) – WBC-adjusted: β = −0.42 y (p=0.05) PA: no DNAmGrimAA effect (β = +0.09 y; p=0.73)	No formal pace clock; EML used PA slowed epigenetic mutation accumulation: – DiD (PA vs control): β = −2.06 “years” (p < 0.001), robust to WBC adjustment Diet: no EML effect (β = −0.37; p=0.39)	NR (no explicit adverse event or safety data reported)	(RoB 2) Low risk for randomization and minimal missing DNAm data, but some concerns due to the open-label lifestyle intervention, unclear blinding, and secondary outcomes with modelling

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																				flexibility.– Low risk: randomization, minimal missing DNAm data – Some concerns: open-label lifestyle, unclear blinding, secondary outcomes with modelling flexibility

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12	Orr, Miranda E	2024	A randomized placebo-controlled trial of nicotinamide riboside in older adults with mild cognitive impairment.	USA	Human	10-week double-blind RCT, NR vs placebo, n=20 analyzed	20	NR 10, placebo 10	Adults ≥65 y with mild cognitive impairment (MoCA<26); mostly Hispanic; mean age ~75–77	Exogenous compound–based	Nicotinamide riboside (NIAGEN®) 1 g/day	Escalation 250→1000 mg/day; total 10 weeks	Placebo	matched capsules, identical escalation	IEAA, EEAA, PhenoAge, GrimAge	PBMCs	Baseline, 10 weeks	No significant within-group or between-group changes in any epigenetic-age metric Exploratory bootstrap: AgeAccelPheno & Grim: subtle decrease with NR; placebo ≈0 (Pheno) or slight ↑ (Grim) EEAA (Hannum): slight increase (accelerated aging) with NR	NR	Mild–moderate AEs similar in NR vs placebo (7/10 each); one placebo stroke; one NR severe nausea resolved with dose reduction	(RoB 2) Some concerns due to the small sample size (n=10 per group), short study duration, and exploratory testing across multiple epigenetic clocks.

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																	IEAA: no change Overall: very small, inconsistent, non-significant effects			
25	Fuentealba, Matias	2025	Multi-Omics Analysis Reveals Biomarkers That Contribute to Biological Age Rejuvenation in	USA	Human	Single-site single-blinded randomized placebo-controlled 4-arm trial; exploratory biological aging	42 completed (DNA methylation analyzed for all)	TPE+IVIG 10; Bi-weekly TPE 11; Monthly TPE 11; Sham 10	Healthy adults ≥50 y (one in 40s allowed), no major clinical disease; exclusions for cardiovascular/pulmonary disease, active cancer/infection, GH/stem	Blood-derived	Therapeutic plasma exchange (1× plasma volume, 5% albumin replacement) ± 2 g IVIG; devices: Spectra Optia; sham procedure with	– Bi-weekly TPE+IVIG: 2 sessions first week of each month × 3 months (6 TPE sessions total) – Bi-weekly TPE (no IVIG):	Placebo / sham-controlled	Sham apheresis with realistic noise/flow simulation; participants, caregivers, and raters blinded	~35 TruAge clocks: Horvath, Hannum, PhenoAge, GrimAge, SystemsAge family, fitness clocks (FitAge, Gait, Grip, VO2max), PC clocks (PCGrimAge, PCHorvath/Hannum/PhenoAge), organ/system clocks (immune, inflammatory, metabolic, kidney, liver, heart, musculoskeletal), stochastic/drift clocks	Peripheral whole blood (EPIC 850k)	Baseline (tp1), before 4th session (tp2), before 6th session (tp3)	At timepoint 2 (peak effect): – TPE+IVIG: −2.61 y (FDR 6.2e-5) vs sham – Monthly TPE: −1.32 y (FDR 2.4e-2) vs sham – Bi-weekly TPE: negative direction; significant vs sham (mean not specified)	NR	1 mild allergic reaction to albumin (0.42%); two total AEs requiring discontinuation (one IVIG-related). No	(RoB 2) Some concerns: allocation was first-come-first-served, raising high concern for sequence generation and

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			Response to Single-Blinded Randomized Placebo-Controlled Therapeutic Plasma Exchange.			endpoints; primary aim = safety & feasibility; 3-month intervention, 3 timepoints			cells, psychiatric illness, anti-aging supplements (1 exception taking rapamycin)		water-filled lines + 250 mL saline	same schedule – Monthly TPE: 1 session/ month × 6 – Sham: identical appearance, no plasma removal						Timepoint 3: No significant differences vs sham (attenuation/compensation) Clock families: SystemsAge immune/inflammatory clocks show largest effects (≈7–10 y decreases in TPE+IVIG; ≈2.5–5 y in monthly TPE) Overall: 15 clocks show significant rejuvenation at tp2 across active arms		major events; well tolerated	possible baseline imbalance; however, blinding of patients, caregivers, and raters was reported, missing data were low (42/44 completed), and outcomes were measured with objective arrays

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																				likely analysed blinded, while testing multiple clocks (35+) raises concerns about multiplicity and selective reporting.
27	Hernández-Arciga, Ulalume	2025	Dietary methionine restriction started late in life promotes	Norway	Human and mouse (Only human	Double-blind randomized 8-week dietary RCT (SAAR vs	20 SAAR participants (paired baseline + 8-	Single analyzed group: SAAR/MetR n=20 (17F / 3M); no control methylation data	Overweight/obese but otherwise healthy adults; mean age 32.9 ± 6.1 y, BMI 31.5 ± 2.3 kg/m²;	Lifestyle	Dietary sulfur amino acid restriction (human analogue of methionine	8 weeks; SAAR vs high-SAA control in parent RCT, but only SAAR	None for epigenetic outcomes (control arm not analyzed)	N/A — only SAAR arm methylation measured; therefore effects are	Universal mammalian clocks (Horvath multi-species panel); mammalian blood clock; other mammalian composite clocks (exact names not fully itemized but derived from	Whole blood (EDTA)	Baseline and 8 weeks	No effect – Authors explicitly state: “8 weeks of the sulfur amino acids diet did not affect the epigenetic age.”	NR	NR (no explicit adverse event or safety data reported)	(RoB 2) Some concerns because only the SAAR arm was analysed,

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			healthy aging in a sex-specific manner.		part was taken)	high-SAA control), but this paper analyzes only a subset of the SAAR arm (n=20) with pre/post DNAm; no control-arm DNAm presented → effectively a single-arm	week samples)		non-smoking, diet-controlled living conditions per parent trial		restriction)	analyzed here		within-group pre–post	Epigenetic Clock Development Foundation)*			– No mean Δ reported; visual inspection shows no significant shift across mammalian clocks. – Direction = null, magnitude = not detectably different from 0.			undermining the randomized comparison, and the reasons for missing methylation data (available for 20/31 SAAR participants) were not described. Measurement risk is low given

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						pre–post epigenetic study.														objective lab-based clocks, and transparent reporting of a null result reduces concerns about selective reporting.
43	McGee, Kirsty C	2024	A combination nutritional supplement reduces DNA methy	UK	Human	Uncontrolled open-label pre–post 12-week intervention; no	Blood: 79; Saliva: 75 (paired baseline + 12-	Single arm only (supplement group; 80 completers)	Healthy older adults ≥60 y; mean age 71.9 ± 6.2 y; BMI 25.8 ± 3.9; 49F / 31M; mostly White	Exogenous compound–based	Daily combination supplement (vitamins + polyphenols + omega-3)	12 weeks daily; ingredients/day: Vit D3 20 µg; Niacinamide 50 mg; Vit C 85 mg;	None (no placebo or control)	N/A – single-arm pre/post only	Blood: Horvath, Hannum, PhenoAge, GrimAge, Mean EpiAge (composite) Saliva: InflammAge (age & acceleration)	Whole blood (EPIC 850K)	Baseline and 12 weeks	Effect_on_DNAmAge — whole cohort: Blood clocks: No significant changes in Horvath, Hannum, PhenoAge, GrimAge, or Mean	NR	NR (no explicit adverse event or safety data reported)	(RoB 2) High risk because there was no randomization or control group,

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			lation age only in older adults with a raised epigenetic age.			comparator arm; DNAm age (blood + saliva) + inflammation + function	week)					Omega-3 (EPA+DHA) 250 mg; Olive extract (10 mg hydroxytyrosol); Resveratrol 30 mg; Astaxanthin 3.2 mg						EpiAge; no significant change in epigenetic age acceleration. Effect_on_DNAmAge — subgroups: Raised Horvath acceleration (≥2 y, n=23): Horvath EAA ↓ ~1.98 y (−21.5%), p=0.069 (trend)			creating high susceptibility to regression to the mean and time effects; the study used an open-label design, included subgroup analyses with multiplicity concerns, and although

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																				missingness was small, the reasons were not detailed.
54	Sae-Lee, Chanachai	2018	Dietary Intervention Modifies DNA Methylation Age Assessed by the Epigenetic Clock.	Netherlands	Human	Double-blind, randomized, placebo-controlled 2-year RCT in older adults, but this epigenetic analysis	44 (all in folic acid + B12 group; paired baseline + 2-year samples)	By sex: 19 males, 25 females	Community-dwelling older adults aged 65–75 y, generally healthy, non-smokers, not heavy drinkers	Exogenous compound–based	Folic acid + vitamin B12 supplementation	Folic acid 400 µg/day + vitamin B12 500 µg/day, orally, daily for 2 years	None for DNAm analysis (placebo arm not used)	Within-group comparison: baseline vs 2-year follow-up in supplemented individuals; analyses stratified by MTHFR C677T genotype (CC vs	Horvath 2013 pan-tissue DNAmAge (age acceleration residual)	Whole blood	Baseline and 2 years	Whole supplemented group (n=44): – Age acceleration residual: no significant change – Mean Δ ≈ −0.77 ± 1.44 years; p = 0.60	NR	NR (no explicit adverse event or safety data reported)	(RoB 2) Some concerns: although the parent trial was a well-conducted double-blind RCT, the epigenetic analysis

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						includes only the supplemented arm (n=44) → effectively single-arm pre–post DNAm with sex × MTHFR genotype stratification; secondary analysis								TT) and sex							included only the active/supplemented arm, losing the randomized comparison. Multiple small-n subgroup analyses (sex × genotype) with one positive finding (female 677CC) raise multipli

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																				city/chance-finding concerns, while objective DNAm measures and minimal missing data suggest low risk for measurement and attrition.
65	Salas-Huetos, Albert	2021	Sperm DNA methylation changes after short-	Spain	Human	14-week randomized controlled parall-l-	72 (paired baseline + 14-week	≈35–37 per arm (nuts vs control; exact split not essential for clock	Healthy men ~18–35 y, consuming Western-style diets; BMI	Lifestyle	Mixed tree-nut supplementation	60 g/day nuts (30 g walnuts + 15 g almonds + 15 g hazelnut	Active dietary control (Western diet witho	Control group maintained habitual Western diet and were	Sperm-specific germline epigenetic age predictor (Jenkins sperm clock); includes epigenetic age and age acceleration	Ejaculated sperm (purified sperm	Baseline and 14 weeks	No effect – No significant within-group change in sperm germline age for	NR	NR (no explicit adverse event or safety data	(RoB 2) Some concerns: the parent randomization was

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			term nut supplementation in healthy men consuming a Western-style diet.			group diet trial; this paper is a pre-planned methylation substudy of ejaculated sperm; ~72 men with paired DNAm data; germline epigenetic aging	sperm methylation)	outcomes)	normal/overweight; no fertility problems; no nut allergies			s) for 14 weeks, consumed atop habitual diet	ut nuts)	instructed not to consume nuts		fraction)		either nuts or control – No significant between-group difference in Δ germ line age – Authors explicitly state: no detectable effect on sperm epigenetic aging, despite many nut-related DMRs		reported)	sound, but the methylation subset (~72/119) may not be fully representative, and participants were unblinded in the diet trial. Measurement bias is likely low due to objective sperm DNAm arrays,

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						assessed														and the methylation outcomes were prespecified with a transparently reported null result.
67	Kou, Minghao	2025	Epigenetic Age Acceleration and Cardiometabolic Biomarkers in Response to Weight-Loss	USA	Human	12-month parallel-arm RCT (low-carb vs low-fat). This Aging Cell paper is a second	Baseline 144; 3 months 129; 12 months 112	Baseline: 71 LF / 73 LC 3 mo: 62 LF / 67 LC 12 mo: 54 LF / 58 LC	Adults 22–75 y with obesity (BMI 30–45); mean age ~47 y; ~89% women; predominantly White and African-American; no	Lifestyle	Low-carbohydrate diet vs low-fat diet	• Low-carb: <40 g/day digestible carbohydrate • Low-fat: <30% energy from fat; <7% saturated fat	Active comparator (two diet arms)	Low-carb vs low-fat; no calorie targets for either arm	PCPhenoAge (AA), PCGrimAge (AA), DunedinPACE	Whole blood (EPIC 850K)	Baseline, 3 months, 12 months	No substantial diet-specific effect on epigenetic aging • DunedinPACE: ~1.00 → 0.99 (very small decrease; similar in both diets) • PCPhenoAge	DunedinPACE decreased slightly (~0.01), but not different by diet; considered negligible effect size	NR (no explicit adverse event or safety data reported)	(RoB 2) Some concerns: the randomized RCT design supports low risk for randomization, but the open-label

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			Dietary Interventions Among Obese Individuals: The MACRO Trial.			ary analysis of DNAm-based biological aging + cardiometabolic biomarkers at 0, 3, 12 months.			diabetes or CVD			Duration 12 months						AA: little change; moves with chronological aging • PCGrimAge AA: similar minimal change; no diet group separation • No significant group×time interactions for any clock			diet raises concerns about deviations, and 12-month attrition (144→112) was not fully explored for prognostic imbalance. Objective DNAm arrays reduce measurement bias, yet as a seconda

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																				ry analysis using multiple clocks, multiplicity remains a concern.
72	Carreras-Gallo, Natalia	2025	Effects of a natural ingredients-based intervention targeting the hallmarks of aging on epigenetic clocks, physic	USA	Human	12-month single-arm, pre–post clinical trial (no randomization, no placebo); 1–4 DNAm measurements per	51 participants (baseline + 3m + 6m + 12m variable availability)	Single cohort (no comparator arms)	Adults ≥55 y; 49% female; age 54–84; relatively healthy with low baseline EAA compared to external aging-biobank reference; self-selected supplement users	Exogenous compound–based	"Cel System" (Cel1, Cel2, Cel3) + 10-min walking + 5-min mindfulness daily	Daily supplementation × 12 months – Cel1: 2-HOBA (hobamine), astragalus extract, rutin, vitamin C, levomefolate, B12, zinc,	None (pre–post only)	Each participant compared to own baseline	– First-generation / PC clocks: PCHorvath pan-tissue, PCHorvath skin&blood, PCHannum, IntrinClock, stochastic clocks – Second-generation: PCPhenoAge, PCGrimAge, OMICmAge, MarioniCAge, DNAmTL – Causal-framework clocks: DamAge, CausAge, AdaptAge – Third-generation pace: DunedinPACE	Whole blood (EPIC 850K)	Baseline, 3 months, 6 months, 12 months	PC Horvath pan-tissue EAA 0.60 → −0.15 at 12m (Δ ≈ −0.75 y; p=0.048) Strongest reduction at 6m: −0.36 ($p=6.1\times10^{-4}$) PC Horvath skin & blood EAA −1.23 → −0.31 (Δ ≈ +0.92 y "older"; p=0.045)	Dunedin PACE 0.94 → 0.99 at 12m (Δ ≈ +0.05, ~5% faster pace; $p = 7.4\times10^{-5}$) Short-term slowing at 3m (0.96) but rebound and overshoot by 12m	NR (no explicit adverse event or safety data reported)	(ROBINS-I) High risk because the study had no randomization, control group, or blinding, and the multi-component

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			al function, and body composition: a single-arm clinical trial.			participant						seleniu m – Cel2: NMN, pterostilbene, astaxanthin, L-carnosine, vitamin D, riboflavin – Cel3: Apigenin, fisetin, oleuropein, EGCG, berberine, ALA, withaferin A Exact pill counts/frequenc			– SystemsAge (overall + organ-specific: lung, immune, metabolic, etc.) – Fitness clocks: DNAmFitAge, DNAmGrip, DNAmGait, DNAmVO2max, DNAmFEV1			PCHannum EAA Worsening at 6m (−0.45 → +0.29; p=0.027), resolves by 12m (−0.15; p=0.80) DamAge (damage-related “causal” clock) 2.46 → −0.66 (3m) and −0.74 (6m) (p≈0.003–0.0014) Partial rebound by 12m: −0.12 (p=0.12) SystemsAge overall			supplement plus lifestyle intervention makes attribution to any single component impossible. Additional concerns include possible selection bias from self-selected supplement users,

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												y not reported						−0.72 → −0.49 (ns; p=0.73) SystemsAge-lung 0.56 → −0.45 (Δ ≈ −1.01 y; p=0.0061) Fitness clocks Example: DNAmGripEAA −0.24 → −0.87 (Δ ≈ −0.63 y; p=0.0042)			missing timepoints (1–4 per participant), industry involvement (authors employed by TruDiagnostic/SRW), and the inability to rule out regression to the mean and time effects.

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73	Borsky, Pavel	2025	Human clinical trial of plasmapheresis effects on biomarkers of aging (efficacy and safety trial).	Czech Republic	Human	Prospective stratified randomized cross-over (G1 = 8 sessions; G2 = delayed-start 4 sessions) Analyzed primarily as dose–response per plasmapheresis session	41 enrolled → 38 at 9w: 34 completers	G1: 28 allocated G2: 13 allocated	Healthy adult first-time plasma donors 40–60 y; screened per standard blood-donor rules; no major chronic disease; median age ~49.6	Blood-derived	Standard donor plasmapheresis (Haemonetics PCS2)	Plasma volume 570–830 mL/session; anticoagulant citrate (CITRASOL 4%); q≥14 days • G1: 8 sessions (0–18 wks) • G2: 0–9 wks control, then 4 sessions (9–18 wks)	Dose–response (per-session) within a partially cross-over design; no untreated control	Effect modeled as change in clock value per additional session adjusting for age, sex, monocyte %, naive CD4 T-cells	Horvath1, Horvath2, PhenoAge, GrimAge, GrimAge2, GrimAge2_tuned, GrimAge2_calibrated, Hannum, RobustHannum, PC clocks (PC Horvath1/2/Hannum/PhenoAge/GrimAge/DNAmTL), DunedinPACE, GrimAge component surrogates (ADM, B2M, Cystatin C, GDF-15, Leptin, PACKYRS, PAI-1, TIMP-1, COX)	Whole blood (buffy coat DNA)	Baseline, 9 weeks, 18 weeks	Significant positive per-session increases: (Estimate = increase in epigenetic age per plasmapheresis session) DNAmGrimAgeBasedOnRealAge: +0.26 ± 0.05 y/session, $p=5\times10^{-7}$ DNAmGrimAge2BasedOnRealAge: +0.22 ± 0.05, $p=2\times10^{-4}$ DNAmGrimAge2_Tuned: +0.16 ± 0.03, $p=1.26\times10^{-5}$	Dunedin PACE increases with each session (+0.003/session). Cumulative effect over 8 sessions ≈ +0.024 (≈2.4% faster pace), directionally adverse.	One participant discontinued early due to hypotension	(RoB 2) High risk: although stratified randomization was used, there was no non-treatment control and the cross-over design only partly mitigates confounding. The study

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						Duration 18 weeks												DNAmGrimAge2_Calibrated: +0.22 ± 0.05, p=2×10⁻⁴ Hannum DNAmAge: +0.17 ± 0.04, p=2×10⁻⁴ RobustHannum: +0.13 ± 0.03, p=2.42×10⁻⁵ DunedinPACE: +0.003 ± 0.001, p=0.0058 (≈ 0.3% faster pace/session)			was not blinded, had a small sample with attrition, tested many clocks with multiple comparisons, and although the mixed-model dose–response analysis was appropriate, time effects,

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																				procedure stress, and lifestyle confounding cannot be ruled out.
75	Yaskolka Meir, Anat	2021	Lifestyle weight-loss intervention may attenuate methylation aging: the	Israel	Human	18-month randomized controlled lifestyle trial (LF vs MED/LC diets; at 6	120 (completepaired methylation)	Originally 4 groups (LF, LF+PA, MED/LC, MED/LC +PA ≈30 each), but DNAm analyses	Sedentary adults with abdominal obesity or dyslipidemia; mean age 48.6±9.3 y, BMI 30.2±3.3; workplace	Lifestyle	Low-fat (LF) diet vs Mediterranean/low-carb (MED/LC) diet; after 6 months each split into diet	Daily hypocaloric diet for 18 months; PA program moderate-intensity aerobic (after 6 months	Active comparator (LF vs MED/LC; ±PA). Epigenetic results mainly within-	LF vs MED/LC; PA vs no PA; but no significant diet-group effects on DNAm aging.	Primary: Li 2018 240-CpG blood DNAmAge Secondary: Horvath 353-CpG (baseline only)	Whole blood (EPIC 850K)	Baseline and 18 months	Cohort (240-CpG clock) mAge increased +1.1 ± 1.9 years over 18 months (≈ chronological aging). No difference between LF (+1.3 y) and	NR	NR (no explicit adverse event or safety data reported)	(RoB 2) Some concerns because the DNAm substudy included only 120/278 particip

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			CENTRAL MRI randomized controlled trial.			months split to ±PA). This Aging Cell paper is a secondary DNAm substudy with n=120 (baseline + 18 months).		pool main diet contrasts (LF vs MED/LC). Subgroup effects (weight-loss success, liver fat status) emphasized.	cohort; 58.8% with fatty liver at baseline.		alone vs diet+PA.	in the PA arms).	subject and by subgroup, not between diets.				MED/LC (+0.9 y), p=0.2. No effect of ±PA.			ants, making selection bias possible, and the open-label lifestyle intervention with variable PA adherence raises concerns about deviations; subgroup findings also increase multiplicity concern

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																				s. Objective methylation assays reduce measurement bias, and there was no selective reporting of diet-group null results.
78	Vetter, Valentin Max	2022	Vitamin D supplementation is associated with	Germany	Human	Prospective two-wave longitudinal cohort (BASE	~1,070 with epigenetic clocks at	• Treated deficient → sufficient: 63 • Untreate	Community-dwelling older adults (60–85 y baseline; 68.3±3.5	Exogenous compound–based	Vitamin D supplementation	Dose, preparation, and adherence not recorded; defined only via	Matched quasi-control groups	Untreated deficient: remained deficient, no supplementation	7-CpG (Vetter) Horvath 2013 Hannum 2013 PhenoAge	Whole blood	DNAm clocks evaluated at follo	Among initially vitamin-D–deficient older adults, those who started supplementat	NR	NR (no explicit adverse event or safety data	(ROBINS-I) High risk of bias because the study

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			slower epigenetic aging.			-II → Gend Age). Quasi-interventional, non-randomized, with optimal pair matching of treated vs untreated vitamin-D–deficient participants.	follow-up (exact n varies slightly by clock).	d deficient: 63 (matched) • Healthy controls (sufficient→sufficient): 63 (matched)	→ 75.6±3.8 at follow-up), ~52% female. Vitamin D deficiency common at baseline (46%).			self-report + medication lists.		Healthy controls: always sufficient, no supplementation. Follow-up ~7.4 years.	GrimAge		w-up (T1). 7-CpG also available longitudinally but intervention effect analyzed cross-sectionally at T1.	ion and became sufficient had about 2.6 years lower 7-CpG DNAm age acceleration and 1.3 years lower Horvath DNAm age acceleration at follow-up than matched deficient non-supplementers, while Hannum, PhenoAge, and GrimAge showed no significant differences.		reported)	was non-randomized with self-selected supplementation, and dose and adherence were not known. Matching only partly reduces confounding, possible lifestyle co-interventions were not

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																				measured, and the use of multiple clocks with subgroup analyses raises concerns about multiplicity.
81	Nwanaji-Enwerem, Jamaji C	2021	An epigenetic aging analysis of randomized metformin and weight loss interv	USA	Human	6-month, 2×2 factorial, randomized, double-blind, placebo-controlled RCT	192	48 per arm	Overweight/obese postmenopausal breast cancer survivors, mean age ~63 y, clinically stable, no active chemo/ra	Exogenous compound–based	Metformin (parent trial: titrated up to ~850 mg BID) Phone-based weight-loss program (calorie restrictio	Metformin daily for 6 months Weight-loss coaching throughout 6 months	Placebo (for metformin) and active lifestyle comparators (factorial design)	Placebo tablets + no weight-loss program (true control arm)	Hannum EAA, Horvath EAA, SkinBlood EAA, IEAA, EEAA, PhenoAge EAA, GrimAge EAA, DNAmTL, EpiTOC, EpiTOC2, MiAge	Peripheral blood (buffy coat)	Baseline and 6 months	Weight loss only vs placebo: • Hannum EAA: +0.98 y (p=0.19) • Horvath EAA: +0.74 y (p=0.42) • SkinBlood EAA: +0.86 y (p=0.21) • PhenoAge EAA: +2.02 y	NR	NR (no explicit adverse event or safety data reported)	(RoB 2) Some concerns because the epigenetic analysis included only 192/333 randomized

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			ention s in overw eight postm enopa usal breast cancer surviv ors.			Arms (each n=48 in EA subset): Metfor min only Weigh t-loss only Weigh t-loss + metfor min Placeb o only Epigen etic clocks are a post-hoc second			diotherap y.		n + PA target ~300 min/wee k)							(p=0.05) — nominal, opposite direction, not interpreted as real • GrimAge EAA: +0.76 y (p=0.27) Metformin only vs placebo: All EAA differences −0.91 to +0.82 y (all p ≥ 0.18). Weight loss + metformin vs placebo: All EAA differences −0.63 to +0.45 y (all p ≥ 0.50).			participants, so the subset may be subject to selectio n bias despite the rigorou s factorial RCT design. Testing many clocks across many contrast s raises multipli city concern s, though objectiv

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						ary analysis.														e EPIC measures reduce measurement bias and consistently reported null results lower selective reporting concerns.
82	Clement, James	2022	Umbilical cord plasma concentrate has beneficial	USA	Human	Phase I single-arm, open-label pre–post trial	18 (paired baseline & post-treatment)		Adults 60–95 y (mean ~74), generally in age-typical health; some with hypertens	Blood-derived	Human umbilical cord blood plasma concentrate (hUCBP secretome)	• 1 mL IM injection weekly × 10 weeks • Each vial derived from 100	None	Pre–post within-subject (baseline vs 10 wks)	Horvath 2013, Hannum 2013, Skin&Blood 2018, PhenoAge, DNAmTL, DNAmGrimAge	Whole blood	Baseline and post-treatment (10 weeks)	Primary signal GrimAge acceleration: 0.04 → −0.78 years → Δ = −0.82 years, p =	NR	2 participants had mild injection-site redness/heat, resolve	(ROBINS-I) High risk because there was no control group, leaving

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			effects on DNA methylation GrimAge and human clinical biomarkers.			10 weekly intramuscular injections of umbilical cord plasma concentrate; outcome measures before vs after			ion, hyperlipidemia, diabetes; 1 with early dementia & RA. No active cancer. 10 women, 8 men.			mL cord plasma (≈5 mg pellet of concentrated extracellular vesicles/ proteins resuspended in 1 mL saline) Total exposure ≈ 1 L cord plasma equivalent						0.0093 (paired t-test) GrimAge protein surrogates: • DNAm Cystatin C ↓ (p = 0.024) • DNAm GDF-15 ↓ (p = 0.0024) Other clocks Horvath, Hannum, Skin&Blood, PhenoAge, DNAmTL: No significant changes		d with diphenhydramine; tolerated further injections	high confounding and regression-to-the-mean concerns, and the sample was very small (N=18) and convenience-based rather than population-based. The study was open-

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																					label with industry involvement, tested multiple clocks and biomarkers, and the GrimAge effect was only nominal (uncorrected).
88	Campisi, Manuela	2025	Unveiling the geroprotective potential of Monar	Italy	Human	Double-blind placebo-controlled randomized	81	Monarda 40; Placebo 41	Adults aged 45–65, university employees undergoing occupatio	Exogenous compound–based	Monarda didyma L. extract	100 mg/day oral capsule for 12 weeks	Placebo	Identical 100 mg maltodextrin capsule daily for 12 weeks.	5-CpG DNAmAge (ELOVL2, C1orf132, KLF14, TRIM59, FHL2)	Whole blood	Baseline and week 12	DNAmAge remained stable in intervention (p=0.45) and increased in placebo (p<0.0001); DNAmAge	NR	NR (no explicit adverse event or safety data reported)	(RoB 2) Some concerns: although the study used a random

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			da didyma L.: insights from in vitro studies and a randomized clinical trial on slowing biological aging and improving quality of life.			parallel-group trial			nal health checks, generally healthy, non-smokers, free of major chronic disease.									significantly lower in intervention vs placebo at week 12 (p=0.016).			ized, double-blind, placebo-controlled RCT design with objective biomarkers, concerns remain due to industry funding, multiple endpoints, modest sample size, and short

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																				duration.
92	Loh, Kah Poh	2023	Exercise and epigenetic ages in older adults with myeloid malignancies.	USA	Human	Single-arm, pre–post pilot trial; ~8–12 weeks of exercise during chemotherapy. DNAm measured at baseline and post-intervention (after 2	20 (complete paired DNAm data)		Adults ≥60 with myeloid malignancies receiving outpatient chemotherapy (AML 55%, MDS 40%, MDS/MPN 5%); mean age 71.2 y, 65% male, 90% White; mixed chemo stages (HMA±venetoclax, others).	Lifestyle	GO-EXCAP (mobile-health–supported home exercise)	Low–moderate intensity progressive walking + resistance bands; daily step tracking via Garmin; ~26 min/day of resistance work; ~3 days/week; duration 8–12	None (pre–post only)	Baseline vs post-intervention within-subject comparison	Horvath, Hannum, IEAA, EEAA, GrimAge, PhenoAge, DunedinPACE	Whole blood	Baseline and 8–12 weeks	• GrimAge: mean Δ −0.7 y (median −1.4), p = 0.17 • PhenoAge: mean Δ −0.8 y (median −1.4), p = 0.35 • DunedinPACE: mean Δ +0.02 (median −0.1), p = 0.47 • Horvath/Hannum/IEAA/EEAA: all non-significant (p ≥ 0.43)	Dunedin PACE: Mean Δ +0.02 (ns); median −0.1 (ns). No statistically significant effect.	NR (no explicit adverse event or safety data reported)	(ROBINS-I) High risk because the study was single-arm and uncontrolled, with strong confounding from chemotherapy, disease course, and regression to the

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						chemo cycles). Exploratory; no control group.						weeks (two chemo cycles).								mean, and it had a very small sample (n=20 paired). Multiple exploratory correlations were tested without multiple-testing correction, although objective DNAm measurement

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																				suggests low measurement bias.
96	Petersen, Curtis L	2021	Weight management intervention identifies association of decreased DNA methylation age with improved functional	USA	Human	Single-arm, pre–post 12-week multi-component weight-loss program (diet + aerobic/resistance exercise); pilot substudy	16 (paired baseline + 12-week samples)		Community-dwelling adults ≥65 y with obesity (BMI >30); mean age 73.5 y, 87.5% women, all White, non-Hispanic.	Lifestyle	12-week structured weight-management program	12 weeks; group sessions; exercise dose not fully quantified (reported adherence data only)	None (pre–post only)	Baseline vs post-intervention within-subject comparison	Horvath, Hannum, PhenoAge (via ENmix)	Whole blood	Baseline and 12 weeks	Mean within-group change (post – pre) • Hannum: −0.8 ± 4.8 y (p = 0.51) • Horvath: −1.1 ± 2.8 y (p = 0.14) • PhenoAge: −0.5 ± 4.1 y (p = 0.64)	NR	NR (no explicit adverse event or safety data reported)	(ROBINS-I) High risk because there was no control group, leaving confounding and regression-to-the-mean concerns, and only 16/28 had

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			age measures in older adults with obesity.			with paired DNAm data.															paired DNAm data, suggesting possible selection bias (those with blood samples had lower baseline grip). The sample was very small with no correction for multiple testing, although

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																				objective DNAm measurement reduces measurement bias.
103	Pavanello, Sofia	2019	Exploring Epigenetic Age in Response to Intensive Relaxing Training: A Pilot Study to Slow Down Biolog	Italy	Human	Prospective before–after pilot (no control). Two groups (both receive intervention): post-MI patients and health	20	14 MI; 6 healthy	Recent MI patients with carotid atherosclerosis (on standard cardiac rehab + meds) and age/sex-matched healthy adults; Caucasian; smokers more common among MI at baseline	Lifestyle	Relaxation Response (RR) training	• 4 days supervised RR sessions • Then 20 min twice daily (morning + evening) at home for 60 days • Both MI and healthy subjects received	None (pre–post only)	Baseline vs post-intervention within-subject comparison	5-CpG DNAmAge (Zbiec-Piekarska model; ELOVL2, C1orf132, KLF14, TRIM59, FHL2)	Whole blood	Baseline (T0) and 60 days (T1)	Mean change (T1–T0) • All subjects: −1.50 ± 4.36 y (p = 0.143) • MI patients: −0.14 ± 2.88 y (p = 0.428) • Healthy subjects: −4.67 ± 5.78 y (p = 0.053)	NR	NR (no explicit adverse event or safety data reported)	(ROBINS-I) High risk because there was no control group and MI patients were also undergoing cardiac rehabilitation and medicat

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			ical Age.			y subjects. Duration: 60 days of twice-daily relaxation practices (after 4 supervised training days).			but stopped after MI.			identical protocol									ion changes, so time effects and confounding cannot be separated. The sample was extremely small (especially the healthy group, n=6), results relied on borderline p-values without multiplicity

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																					correction, and although objective DNAm and qPCR assays reduce measurement bias, these design limitations remain substantial.
107	Wang, Shuyue	2025	Nucleotides as an Anti-Aging Supplementation	China	Human	19-week, double-blind, randomized, placebo-	121	59 NTs, 62 placebo	Community adults 60–70 y, generally healthy; ~66% female. Balanced	Exogenous compound–based	Mixed 5′-NTs supplementation	• 1.2 g/day total NTs (4 × capsules/day) • Each capsule:	Placebo control	Placebo capsules (0.4 g starch)	PC-corrected Horvath, Hannum, GrimAge, PhenoAge, plus Median DNAmAge = clock-median of the four PC ages (primary)	Whole blood	Baseline (T0), week 11 (T1), week	NTs vs placebo Δ at 19 weeks: β = −3.08 years (95% CI −5.07, −1.10), p = 0.0023	NR	Transient rise in uric acid at week 11 (+31.6 μmol/L,	(RoB 2) Overall low risk: randomization, allocation

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			in Older Adults: A Randomized Controlled Trial (TALENTs study).			controlled parallel RCT			comorbidities (HTN 30%, diabetes 14%, CVD 54%, renal disease 40%). Extensive COVID-19 infections during trial (~75–80% both arms by midpoint).			0.3 g NTs + 0.1 g starch • Composition (modeled on breast milk): AMP:CMP:GMP:UMP = 16:41:19:24 • Duration 19 weeks					19 (T2)	→ ~3-year epigenetic age reduction vs placebo over ~4.5 months. Midpoint (week 11): β = −1.94 y (p = 0.11) — trend only.		p<0.001), attenuated at week 19	concealment, and blinding were appropriate, attrition was very low with ITT GEE analyses, and objective molecular outcomes were assessed by blinded lab staff, limiting measur

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																				ement bias. Some concerns remain due to sponsor involvement (supplement provided), while COVID infections may add noise but were balanced across arms.

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118	Gensous, Noemie	2020	One-year Mediterranean diet promotes epigenetic rejuvenation with country- and sex-specific effects: a pilot study from the NU-AGE project.	Italy and Poland	Human	1-year randomized controlled dietary intervention (NU-AGE RCT), but epigenetic substudy includes only intervention arm → uncontrolled pre–post design for	120	Single group	Older adults 65–79 y, community-dwelling, generally healthy (no cancer, severe organ disease, dementia, frailty); Italy 27M/33F; Poland 24M/36F	Lifestyle	NU-AGE individualized Mediterranean-style diet: high vegetables, fruits, legumes, whole grains, olive oil; low red meat; age-tailored micronutrients	12-month continuous dietary counseling + adherence monitoring (NU-AGE score ↑ from ~52 to 65–67)	None for epigenetic outcomes (control arm not included in methylation analysis)	N/A — pre–post only	Horvath DNAmAge (pan-tissue) and its acceleration measures: AgeAccel, IEAA (intrinsic), EEAA (extrinsic)	Whole blood	Baseline (T0) and 12 months (T1)	AgeAccel Italy (all): NS (p=0.182) Italy males: NS trend (p=0.063) Italy females: NS Poland (all): −AgeAccel (p=0.031; adj p=0.094) Poland females: significant rejuvenation → p=0.0013; BH adj p=0.008 Poland males: NS IEAA	NR	NR (no explicit adverse event or safety data reported)	(RoB 2) High risk for causal interpretation because the methylation analysis lacked a control arm, appears limited to the intervention arm (possible selection bias), used small subgroups, and involve

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						DNAm outcomes												Italy (all): small decrease (p=0.035; adj p=0.104) Poland females: significant decrease (adj p=0.042) Others: NS EEAA No significant effects in any subgroup			d multiple tests with modest effect sizes. Measurement bias is likely low given objective array-based assays.
123	Obeid, R	2018	Effect of adding B-vitamins to vitamin D and	Germany	Human	12-month double-blind randomized controlled trial;	63	D+Ca: 31 D+Ca+B: 32	Older adults, mean age 68.4 ± 10.1 y; majority female (imbalance toward	Exogenous compound–based	Vitamin D3 + Calcium + B-vitamins (folate, B6, B12)	Daily × 12 months – Both arms: Vitamin D3 1200 IU/day, Calcium	Active control	Vitamin D3 + Calcium only (B-vitamin capsules replaced with	Weidner 3-CpG clock (ASPA, ITGA2B, PDE4C) via pyrosequencing	Whole blood	Baseline and 12 months	CpG-specific methylation changes ASPA (normally decreases with age):	NR	NR (no explicit adverse event or safety data reported)	(RoB 2) High risk for epigenetic aging inference due to the

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			calcium supplementation on CpG methylation of epigenetic aging markers.			two arms (D+Ca vs D+Ca+B); this is a secondary epigenetic analysis (n=63) using targeted CpG methylation and the Weidner 3-CpG clock			B-vitamin group); community-dwelling; screened to exclude major illness or medication changes			carbonate 800 mg/day – B-vitamin arm only: Folic acid 0.5 mg, Pyridoxine 50 mg, Cyanocobalamin 0.5 mg (3 capsules/day)		inert filler)				– D+Ca: −0.96% – D+Ca+B: +1.40% → significant between-group effect (p=0.046) → younger PDE4C (normally increases with age): – D+Ca: +0.22% – D+Ca+B: +1.95%, trend (p=0.062) → older			small sample, sex/age imbalance between arms, and reliance on a semi-validated 3-CpG clock with internal calibration (risk of circularity). Additional concerns include lower pyroseq

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																				uencing precision for PDE4C, small effect sizes, and borderline p-values.
148	Horvath, Steve	2025	Cognitive rejuvenation in old rats by hippocampal OSKM gene therapy.	USA and Argentina	Rat (female Sprague–Dawley)	In vivo gene-therapy intervention; 3 groups: young intact, old control (GFP vector), old OSKM	20 hippocampal samples (6 young, 6 old control, 8 old OSKM)	Young 6; Old control 6; Old OSKM 8	Female SD rats; young 3.5 mo; old 25.3 mo; standard housing; ad libitum food/water	Celular reprogrammation	Bilateral stereotaxic delivery of OSKM-GFP high-capacity adenovector (Tet-Off) into dorsal dentate gyrus	Single bilateral 3 µL injection per hemisphere; OSKM expression for 39 days; GFP-only vector for controls	Active (GFP-only) + age comparison to young intact	Old controls received identical surgery and GFP-only vector; young rats left intact	Rat brain DNAmAge clock; human–rat relative age clock; mouse brain clock (MammalMethylChip40)	Hippocampus	Single terminal measure after 39 days	All comparisons: old OSKM vs old controls Rat brain clock – DNAmAge slightly younger in OSKM vs control – p = 0.064 (two-sided), p < 0.05 one-sided	NR	– No seizures, teratoma-like lesions, or behavioral toxicity observed – Long-term safety unknown	(RoB 2) High risk (exploratory) because allocation was not clearly randomized, blinding of behavioral scoring was not

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						vector; ~39-day expression; behavioral + DNAm outcomes; non-randomized, parallel-group												Human–rat relative age clock – OSKM slightly younger – p = 0.076 (two-sided) Mouse brain clock – OSKM slightly younger – p = 0.088 (two-sided) Estimated magnitude: <1 “rat-year”.			reported, sample sizes were small, and the study involved multiple hypothesis testing (clocks, chromatin states, EWAS) over a short duration.

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149	Horvath, Steve	2023	Reversal of Biological Age in Multiple Rat Organs by Young Porcine Plasma Fraction.	India (treatment), with collaborators in USA, Argentina, Croatia	Rat (male Sprague–Dawley; replication includes both sexes)	Main experiment: non-randomized 3-arm parallel-group (young vs old control vs old + E5); 155-day intervention with terminal multi-organ DNAm	Main experiment: 6 young, 6 old control, 6 old + E5 (multi-organ) Replication: 9 E5 vs 8 saline (blood)	Young 6; Old control 6; Old + E5 6 (main) E5 9; Saline 8 (replication)	Old male SD rats (109 weeks ≈ 2+ years old); young reference 30 weeks; replication cohort 26-month mixed-sex SD rats	Blood-derived	E5 — exosome-rich plasma fraction from 6-month-old pigs (Yorkshire breed)	Two IV series, each consisting of 4 injections on alternate days (8 total), separated by 95 days; tail-vein administration.	Active (old untreated control) + age reference (young)	Old control rats received saline; young rats untreated	Rat pan-tissue clock; rat blood clock; rat liver clock; rat brain clock; human–rat pan-tissue clocks (absolute & relative age)	Whole blood	Single terminal time point at day 155 (main); baseline + day 15 (replication)	Liver: −77.6% epigenetic age Blood: −68.2% Heart: −56.5% Hypothalamus: −29.6% Mean across 4 organs: −67.4% (≈ halving of biological age) Clock ranges (original models): liver 68.6–78.6%; blood 52.5–74.5%; heart 46.5%; hypothalamus 24.4%. Replication experiment	NR	No overt toxicity observed over 155 days	(RoB 2) High risk (exploratory) because the main study was not explicitly randomized or blinded, group sizes were very small (n=6), there were many endpoints with multiple compari

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						profiling Replication experiment: randomized E5 vs saline in 26-month-old rats (blood DNAm only)												(baseline → day 15) 26-month-old rats: Rat blood clock: significant rejuvenation (p = 0.0094) Rat pan-tissue clock: trend (p = 0.054), significant after removing 1 outlier (p = 0.00086); significant in females (p = 0.014)			sons, and generalizability is limited by species specificity. Despite this, the reported effects were large, consistent, and replicated across tissues, clocks, and a second cohort.

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150	Chiavellini, Priscila	2024	Young Plasma Rejuvenates Blood DNA Methylation Profile, Extends Mean Lifespan, and Improves Physical Appearance in Old Rats.	Argentina	Rat (female Sprague–Dawley)	Non-randomized 2-arm longitudinal intervention in very old rats, with q2-week blood DNAm until natural death; additional younger reference cohorts for	17 old rats: Control 8, Treated 9 (187 total repeated blood samples)		25.6-month-old female SD rats (late-life), housed standard conditions; age-reference cohorts at 3.7, 8, 15.7 months used for DNAm-age curve only	Blood-derived	Biweekly young-plasma injections (from 2-month-old donors)	1 mL plasma intraperitoneal every 2 weeks, from 25.6 months until death	Untreated old control group	Control rats handled but did not receive plasma	Horvath rat blood DNAm-age clock	Whole blood	Repeated q2-week blood draws from 25.6 months → death; aggregated into age bands 27–31.5 mo and 32.5–35.5 mo for primary	Treated vs control: Immediate post-intervention: DNAm age lower in treated rats at nearly all timepoints (NS per-timepoint). Primary positive finding (age-banded): 27–31.5 mo: NS 32.5–35.5 mo: Significantly lower DNAm age in treated rats (~3–4 months	NR	NR (no explicit adverse event or safety data reported)	(RoB 2) High risk because the study was non-randomized with a small sample, unblinded design, and subjective survival and appearance outcomes, with exploratory DMC analysis

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						clock trajectory											comparison	younger, p<0.05)			; allocation and detection biases are therefore high, although DNAm measurement itself presents low risk.
219	Izquierdo, AG	2025	Epigenetic Aging Acceleration in Obesity Is Slowed Down by	Spain	Human	Mixed design: Cross-sectional (obesity vs normal-	Cross-sectional: 48 (28 obese, 20 normal-weight)		Adults with obesity (BMI>30), mean age ~49 y; European Caucasian; excluded recent major weight	Lifestyle	PNK® Very Low-Calorie Ketogenic Diet (VLCKD), structured 5-stage commerc	6-month diet: strict ketogenic phase → stepwise food reintroduction Assessments:	Pre–post within-subject (no external control)	Baseline vs NK vs EP within same individuals	Horvath, Hannum, Levine PhenoAge Outcome = AgeAccel (DNAmAge − chronological age)	Whole blood	Day 0 (BL), Day 30 (NK), Day 180 (EP)	Cross-sectional (obesity vs normal weight) Obesity: AgeAccel ≈ +4.4 y Normal weight:	NR	NR (no explicit adverse event or safety data reported)	(ROBINS-I) High risk because there was no control group for epigenetic

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			Nutritional Ketosis Following Very Low-Calorie Ketogenic Diet (VLCKD): A New Perspective to Reverse Biological Age			weight) Single-arm longitudinal VLCKD (n=10 obese adults), no control, pre–post at 0 / 30 / 180 days	VLCKD: 10 obese adults		change, meds except antidiabetics; both sexes		ial program	baseline (BL), nutritional ketosis (NK ~day 30), endpoint (EP ~day 180)						AgeAccel ≈ −3.1 y All clocks significantly higher AgeAccel in obesity (p<0.0001) Strong BMI correlation (r=0.76–0.81)			outcomes and the sample was very small (n=10), so regression to the mean and time effects cannot be ruled out. Multiple exploratory metabolic and ketone correlations with

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																					limited multiplicity correction and industry involvement (PNK® advisory relationships) add further bias concerns, although standardized DNAm methods suggest low measur

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																				ement bias.
244	Reading, C L	2025	An exploratory analysis of bezisterim treatment associated with decreased biological age acceleration, and improved	USA and Argentina	Human	Phase 3 randomized, double-blind, placebo-controlled parallel-group trial (30 weeks); epigenetic analysis = explor	33 (17 bezisterim, 16 placebo)	Bezisterim 17; Placebo 16 (per-protocol completers with QC-passing DNAm)	Age 60–85 y; probable AD by NIA-AA 2011; CDR global 1–2; MMSE 14–24; MRI/CT excluding other pathology; balanced baseline demographics and metabolic/cognitive profiles between arms	Exogenous compound–based	Bezisterim (NE3107/HE3286) – 17α-ethynyl-androst-5-ene-3β,7β,17β-triol	20 mg oral BID for 30 weeks (≈210 days)	Placebo	Matching placebo, BID, same schedule and procedures	SBCAge (Horvath Skin&Blood), PhenoAge, GrimAge, Hannum, InflammAge	Whole blood	Single post-treatment sample at completion (day 150–210); no baseline DNAm	Outcome metric: dAge = Biological Age − Chronological Age (years); negative = biologically younger. Values below are bezisterim − placebo: SBCAge: −3.68 y, p = 0.017 PhenoAge: −3.71 y, p = 0.081 (trend)	NR	Any TEAE: Placebo 72.7% (24/33) vs Bezisterim 62.5% (15/24) Headache: more common with bezisterim (12.5% vs 0%)	(RoB 2) Some concerns because the epigenetic subset was small and restricted to selected completers (33/439), potentially healthier or

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			clinical measure and biomarker changes in mild-to-moderate probable Alzheimer's disease.			atory subset with post-treatment single timepoint only (between-group comparison, no baseline DNAm)												GrimAge: −1.92 y, p = 0.068 (trend) Hannum: −5.00 y, p = 0.006 InflammAge: −4.77 y, p = 0.022		Treatment-related TEAEs: Bezisterim 12.5% (3/24) vs Placebo 18.2% (6/33) SAEs: 1 in bezisterim (pneumonia, not related); 7 events in 3 placebo participants (none related)	late-enrolled participants, raising possible selection bias. Measurement bias is likely low given standard EPIC v2.0 assays with explicit batch processing, while BioVie's design and sponsorship

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																			Discontinuations due to AE: 3 in placebo, 0 in bezisterim	may introduce interpretive bias despite transparent, explicitly exploratory analyses.
314	Perlmutter, A	2024	The impact of a polyphenol-rich supplement on epigenetic and cellular marke	USA	Human	Single-arm, open-label pre–post pilot; no control group; 90-day supplementation;	47 with paired DNAm; per-protocol n=40 for primary analysis	Single-arm (n=40 PP)	Generally healthy adults, age 18–85 (mean 54 ± 11 y), BMI 24.2 ± 3.3, 40% male; excluded BMI ≥40, major recent lifestyle changes;	Exogenous compound–based	HTB Rejuvenate (Himalayan Tartary buckwheat–based polyphenol formulation)	4 capsules/day (2 AM, 2 PM with food) × 90 days;	None (pre–post only)	Baseline DNAm → day-90 DNAm within same participants	OMICmAge, PCPhenoAge, PCGrimAge v1, DunedinPACE	Whole blood	Baseline (day 0) and day 90	OMICmAge EAA: NS (p=0.740) PCPhenoAge EAA: NS (p=0.690) PCGrimAge EAA: NS (p=0.320)	Dunedin PACE: No significant change reported	NR (no explicit adverse event or safety data reported)	(RoB 2) High risk of bias due to the open-label design with no comparator, small sample size, subgrou

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			rs of immune age: a pilot clinical study			paired DNAm baseline → day 90			recruited remotely across US											p analyses without multiplicity correction, sponsor involvement, and uncontrolled lifestyle confounding.
350	Zhang, B	2023	Multi-omic rejuvenation and life span extension on exposure to youthf	USA	Mouse (female C57BL/6J)	Long-term heterochronic parabiosis (old 20 mo + young 3 mo) vs old	Typically 5–7 per group p per tissue per platform (RRB		Old female C57BL/6J (20 mo) paired with young 3 mo	Blood-derived	3-month heterochronic parabiosis (vs old–old isochronic)		Old isochronic (O:O)		– RRBS clocks: 2 multi-tissue (Meer, Thompson), 1 blood clock (Petkovich), 1 scAge – Array clocks: Universal relative-age, Universal log-linear, Mouse liver, Mouse liver developmental	Blood and Liver	End of 3 mo attachment; 2 mo post-detachment; short	Blood (post-detachment): ~16–32% younger epigenetic age Liver: • Attached: ~17–27% younger (arrays), ~5–26% (RRBS)	Surgical risk; no long-term toxicity reported	NR (no explicit adverse event or safety data reported)	(RoB 2) High risk (exploratory) due to the preclinical surgical model with

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			ul circulation			isochronic controls; 3 months parabiosis, with attached and 2-month detached cohorts; also 5-week short-term HPB; includes multi-omic profiling +	S & array)										-term 5-week HPB	• Detached: ~11–26% younger (sustained rejuvenation) Short-term 5-week HPB: 0–11% reduction, often non-significant Interpretation: Robust, multi-platform evidence of systemic epigenetic rejuvenation, durable after detachment.			small sample size, but the reported rejuvenation effects were highly consistent across eight clocks, multiple tissues, and omics layers.

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						longevity														
364	Waziry, R	2023	Effect of long-term caloric restriction on DNA methylation measures of biological aging in healthy	USA	Human	24-month randomized controlled trial (2:1 CR vs ad-libitum), post-hoc DNAm aging analysis;	128 CR, 69 AL		Healthy non-obese adults, age 21–50 (men) / 21–47 (women), BMI 22–27.9	Lifestyle	25% caloric restriction (achieved ≈12%) for 2 years		Ad-libitum diet (no CR)		PC PhenoAge, PC GrimAge, DunedinPACE (primary)	Whole blood	Baseline, 12 mo, 24 mo	PC PhenoAge: No CR vs AL difference (d = −0.03 @12 mo; +0.05 @24 mo; NS) PC GrimAge: No difference (d = −0.04 @12 mo; +0.05 @24 mo; NS) DunedinPACE: Significant slowing	Not reported in this paper; parent CALERIE RCT showed acceptable 2-year safety	NR (no explicit adverse event or safety data reported)	(RoB 2) Low to moderate risk: the study used a proper RCT design with a high-quality DNAm assay, but the epigenetic analysis

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			adults from the CALERIE trial			n=197 with DNAm												12 mo: d = −0.29, P < 0.003 24 mo: d = −0.25, P < 0.003 ≈ 2–3% reduction in pace of aging TOT (20% CR): DunedinPACE d ≈ −0.40 to −0.43 (stronger effect)			was post hoc, adherence was low (<25%), and the population was a healthy, selective sample.
436	Horvath, S	2021	DNA methylation age analysis of rapamycin in common	USA	Non-human primate (comm	Chronic rapamycin vs vehicle cross-sectional compa	37	20 control, 17 rapamycin	Middle-aged captive marmosets (~9–10 y), relatively healthy	Exogenous compound–based	Rapamycin ~1 mg/kg/day, oral in yogurt, 5 days/week	~2–3.5 years exposure	Vehicle (Eudragit)		Marmoset blood epigenetic clock (trained in separate 58-sample set)	Whole blood	Single post-treatment sample (no baseline	Multivariate regression (DNAmAge ~ age + sex + treatment): Rapamycin coefficient: −0.18 y, p =	NR	NR (no explicit adverse event or safety data reported)	(ROBINS-I) High risk because the study was non-random

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			marmosets		on marmoset)	rison after ~2–3.5 y exposure; not randomized										DNAm)	0.686 → NS; no effect EWAS: No genome-wide significant CpGs; only 48 CpGs at p<0.005 (exploratory) Rapamycin does not alter blood DNAmAge in these middle-aged marmosets			ized and cross-sectional with no baseline DNAm measurement, limiting causal interpretation, although measurement quality was high.
457	Demidenko, O	2021	Rejuvant®, a potential life-extending	USA	Human	Uncontrolled before–after in supplement custo	42		Healthy adults, mean age ~63 y; low BMI; heavy supplement users	Exogenous compound–based	(Ca-AKG 1 g/day + Vitamin A (men) or D (women))	2 tablets/day for 4–10 months (mean ~7 months)	None		9-CpG targeted Sanger (TruMe "TruAge" proprietary clock)	Saliva	Baseline → ~7 months	Full cohort: Mean TruAge −7.96 y (biological age decrease ≈8 y)	NR	NR (no explicit adverse event or safety data	(ROBINS-I) Very high risk because there was no

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			compound formulation with alpha-ketoglutarate and vitamins, conferred an average 8 year reduction in biological aging, after an average of 7 months of use, in the			mers; no placebo/no randomization												Lifestyle-stable subset (n=13): Mean −7.69 y Stats: One-sided paired t-tests ($p \approx 10^{-5}$–10^{-12}); no control group Very large apparent rejuvenation by proprietary clock; cannot infer causality		reported)	control group, the study relied on a proprietary 9-CpG saliva clock, and there were concerns about selection bias and sponsor involvement.

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			TruAge DNA methylation test																	
509	Wang, T	2017	Epigenetic aging signatures in mice livers are slowed by dwarfism, calorie restriction and rapamycin treatment	USA and UK	Mouse	Preclinical comparison of long-lived models vs age-matched WT; n=4 per group	32	~4/group × 8 groups across ages & interventions	Ames dwarfs (male), UM-HET3 females; 2 mo & 22 mo	Exogenous compound–based	Prop1df/df dwarfism, 40% calorie restriction, rapamycin 42 mg/kg diet (4–22 mo)	Genetic; CR at 60% of ad-lib; rapamycin lifelong (4→22 mo)	Age-matched WT (same strain)		Mouse liver DNAmage (ElasticNet, 148 CpGs)	Liver	2 mo and 22 mo	Ames dwarf: −10.1 months epigenetic age Calorie restriction: −9.4 months Rapamycin: −6.0 months Young dwarfs (2 mo): −1.5 mo (developmentally younger) All significant ($p < 0.05–10^{-4}$)	NR	NR (no explicit adverse event or safety data reported)	(ROBINS-I) Moderate to high risk due to the very small sample size (n=4) and lack of randomization for DNAm outcomes, although the

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																				direction of effects was consistent across all interventions.
515	Reynolds, Lindsay	2025	A tree nut and extra virgin olive oil intervention to improve cardiometabolic health – a feasibility	USA	Human	Single-arm 4-week dietary feasibility study with randomized education vs no-education sub-comparison;	29		Adults 48–81 y (mean 68) with metabolic syndrome (≥3 MetS criteria)	Lifestyle	Daily 1 oz tree nuts + 2 Tbsp EVOO	Nuts 1 oz/day; EVOO 2 Tbsp/day; 4 weeks	Within-subject pre–post (no dietary control)		DunedinPACE, AgeAccelGrim	Whole blood	Baseline, 4 weeks	DunedinPACE: Δ = −0.002 ± 0.070, p = 0.86 (NS) AgeAccelGrim: Δ = −0.04 ± 1.34, p = 0.89 (NS) Between-arm (education vs no education): not analyzed for DNAm outcomes	NR	NR (no explicit adverse event or safety data reported)	(ROBINS-I) High risk because there was no control group, the sample was very small, the duration was only 4 weeks,

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			study incorporating epigenetic aging			no diet control group												Baseline: DunedinPACE mean 1.179 (all >1.0 → accelerated aging in MetS)			and the feasibility design relied on pre–post comparisons only.

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References

1. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. Cell. 2023;186(2):243-278. doi: 10.1016/j.cell.2022.11.001.
2. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The Hallmarks of Aging. Cell. 2013 Jun;153(6):1194–217.
3. Sen P, Shah PP, Nativio R, Berger SL. Epigenetic Mechanisms of Longevity and Aging. Cell. 2016 Aug;166(4):822–39.
4. Braga DL, Mousovich-Neto F, Tonon-da-Silva G, Salgueiro WG, Mori MA. Epigenetic changes during ageing and their underlying mechanisms. Biogerontology. 2020;21:423-443. doi:10.1007/s10522-020-09874-y.
5. Pal S, Tyler JK. Epigenetics and aging. Sci Adv. 2016;2(7):e1600584. doi:10.1126/sciadv.1600584
6. Wang K, Liu H, Hu Q, et al. Epigenetic regulation of aging: implications for interventions of aging and diseases. Signal Transduct Target Ther. 2022;7(1):374. doi:10.1038/s41392-022-01211-8.
7. Ciccarone F, Tagliatesta S, Caiafa P, Zampieri M. DNA methylation dynamics in aging: how far are we from understanding the mechanisms? Mech Ageing Dev. 2018;174:3-17. doi:10.1016/j.mad.2017.12.002.
8. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet. 2018;19(6):371-384. doi:10.1038/s41576-018-0004-3.
9. Belsky DW, Caspi A, Corcoran DL, et al. DunedinPACE, a DNA methylation biomarker of the pace of aging. eLife. 2022;11:e73420. doi:10.7554/eLife.73420.
10. Jones MJ, Goodman SJ, Kobor MS. DNA methylation and healthy human aging. Aging Cell. 2015;14:924-932. doi:10.1111/acel.12349.
11. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14(10):R115. doi:10.1186/gb-2013-14-10-r115.
12. Levine ME, Lu AT, Quach A, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging (Albany NY). 2018;10(4):573-591. doi:10.18632/aging.101414.
13. Lu AT, Binder AM, Zhang J, et al. DNA methylation GrimAge version 2. Aging (Albany NY). 2022;14(23):9484-9549.
14. Ulalume Hernández-Arciga, Ceda Stamenkovic, Yadav S, Nicoletti C, Albalawy WN, Farazdaq Al Hammood, et al. Dietary methionine restriction started late in life promotes healthy aging in a sex-specific manner. Science advances. 2025 Apr;11(16).
15. Salas-Huetos A, James ER, Jordi Salas-Salvadó, Mònica Bulló, Aston KI, Carrell DT, et al. Sperm DNA methylation changes after short-term nut supplementation in healthy men consuming a Western-style diet. Andrology. 2021 Jan;9(1):260–8.
16. Kou M, Li X, Yoriko Heianza, Dorans K, Bazzano L, Qi L. Epigenetic age acceleration and cardiometabolic biomarkers in response to weight-loss dietary interventions among obese individuals: The MACRO trial. Aging cell. 2025 Nov;24(11).
17. Izquierdo AG, Lorenzo PM, N Costa-Fraga, Primo D, G Rodríguez-Carnero, Nicoletti CF, et al. Epigenetic aging acceleration in obesity is slowed down by nutritional ketosis following very low-calorie ketogenic diet (VLCKD): A new perspective to reverse biological age. Nutrients [Internet]. 2025;17(6). Available from: [https://www.scopus.com/inward/record.uri?eid=2-s2.0-105001235593&doi=10.3390%2Fnu17061060&partnerID=40&md5=092fee851bef241ad29428ecbd8ef480]
18. Noémie Gensous, Paolo Garagnani, Santoro A, Giuliani C, Ostan R, Fabbri C, et al. One-year Mediterranean diet promotes epigenetic rejuvenation with country- and sex-specific effects: a pilot study from the NU-AGE project. GeroScience. 2020 Apr;42(2):687–701.
19. Reynolds LM, Howard TD, Langefeld CD, Vitolins MZ. A tree nut and extra virgin olive oil intervention to improve cardiometabolic health – a feasibility study incorporating epigenetic aging. Preprints.org [Preprint]. 2025 Feb 7. doi:10.20944/preprints202502.0506.v1.
20. R Waziry, Ryan CP, Corcoran DL, Huffman KM, Kobor MS, Kothari M, et al. Effect of long-term caloric restriction on DNA methylation measures of biological aging in healthy adults from the CALERIE trial. Nature Aging [Internet]. 2023;3(3):248–57. Available from:

 doi:10.20944/preprints202603.0713.v1

[https://www.scopus.com/inward/record.uri?eid=2-s2.0-85147758471&doi=10.1038%2Fs43587-022-00357-y&partnerID=40&md5=32f05ca439f47daaa689609f53602555]

21. Loh KP, Chandrika Sanapala, Jensen-Battaglia M, Rana A, Sohn MB, Watson E, et al. Exercise and epigenetic ages in older adults with myeloid malignancies. European journal of medical research. 2023 May;28(1):180.
22. Fiorito G, Saverio Caini, Palli D, Bendinelli B, Saieva C, Ilaria Ermini, et al. DNA methylation-based biomarkers of aging were slowed down in a two-year diet and physical activity intervention trial: the DAMA study. Aging cell. 2021 Oct;20(10).
23. Fitzgerald KN, Hodges R, Hanes D, Stack E, Cheishvili D, Szyf M, et al. Potential reversal of epigenetic age using a diet and lifestyle intervention: a pilot randomized clinical trial. Aging. 2021 Apr;13(7):9419–32
24. Meir AY, Keller M, Bernhart SH, Ehud Rinott, Gal Tsaban, Hila Zelicha, et al. Lifestyle weight-loss intervention may attenuate methylation aging: the CENTRAL MRI randomized controlled trial. Clinical epigenetics. 2021;13(1):48.
25. Petersen CL, Christensen BC, Batsis JA. Weight management intervention identifies association of decreased DNA methylation age with improved functional age measures in older adults with obesity. Clinical epigenetics. 2021 Mar;13(1):46.
26. Pavanello S, Campisi M, Tona F, Lin CD, Sabino Iliceto. Exploring epigenetic age in response to intensive relaxing training: A pilot study to slow down biological age. International journal of environmental research and public health. 2019 Aug;16(17).
27. Nwanaji-Enwerem JC, Chung FFL, Van der Laan L, Novoloaca A, Cuenin C, Johansson H, et al. An epigenetic aging analysis of randomized metformin and weight loss interventions: two clinical trials. Clinical Epigenetics. 2021;13(1):180. doi:10.1186/s13148-021-01164-6.
28. Reading CL, Yan J, Testa MA, Simonson DC, Javaid H, Schmunk LJ, et al. An exploratory analysis of bezisterim treatment effects on epigenetic age biomarkers in older adults with mild-to-moderate probable Alzheimer's disease. Aging (Albany NY). 2025;17(4):825-848. doi:10.18632/aging.206282.
29. Horvath S, Zoller JA, Haghani A, Lu AT, Raj K, Jasińska AJ, et al. DNA methylation age analysis of rapamycin in common marmosets. GeroScience. 2021;43(5):2411-2423. doi:10.1007/s11357-021-00434-0.
30. Wang T, Tsui B, Kreisberg JF, Robertson NA, Gross AM, Yu MK, et al. Epigenetic aging signatures in mice livers are slowed by dwarfism, calorie restriction and rapamycin treatment. Genome Biol. 2017;18(1):57. doi:10.1186/s13059-017-1186-2.
31. Bischoff-Ferrari HA, Gängler S, Wieczorek M, Belsky DW, Ryan J, Kressig RW, et al. Individual and additive effects of vitamin D, omega-3, and exercise on biological aging in DO-HEALTH. Nature Aging. 2025;5(3):313-326. doi:10.1038/s43587-025-00792-7.
32. Vetter VM, Sommerer Y, Kalies CH, Spira D, Bertram L, Demuth I. Vitamin D supplementation is associated with slower epigenetic aging. GeroScience. 2022;44(4):1843-1859. doi:10.1007/s11357-022-00542-x.
33. Chanachai Sae-Lee, Corsi S, Barrow TM, Kuhnle GGC, Bollati V, Mathers JC, et al. Dietary intervention modifies DNA methylation age assessed by the epigenetic clock. Molecular nutrition & food research. 2018 Dec;62(23).
34. Obeid R, U Hübner, Bodis M, Graeber S, Geisel J. Effect of adding B-vitamins to vitamin D and calcium supplementation on CpG methylation of epigenetic aging markers. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2018 Apr;28(4):411–7.
35. McGee KC, Sullivan J, Hazeldine J, Schmunk LJ, Martin-Herranz DE, Jackson T, et al. A combination nutritional supplement reduces DNA methylation age only in older adults with a raised epigenetic age. GeroScience. 2024 Oct;46(5):4333–47.
36. Carreras-Gallo N, Dargham R, Thorpe SP, Warren S, Mendez TL, Smith R, et al. Effects of a natural ingredients-based intervention targeting the hallmarks of aging on epigenetic clocks, physical function, and body composition: a single-arm clinical trial. Aging. 2025 Mar;17(3):699–725.
37. Demidenko O, Barardo D, Budovskii V, Finnemore R, Palmer FR, Kennedy BK, et al. Rejuvant®, a potential life-extending compound, reduces the epigenetic age in several cell types. Aging. 2021;13(20):24008-24030. doi:10.18632/aging.203643.

 doi:10.20944/preprints202603.0713.v1

38. Perlmutter A, Bland JS, Chandra A, Malani SS, Smith R, Mendez TL, et al. The impact of a polyphenol-rich supplement on epigenetic and cellular markers of immune age: a pilot clinical study. Frontiers in Nutrition [Internet]. 2024;11. Available from: [https://www.scopus.com/inward/record.uri?eid=2-s2.0-85211133065&doi=10.3389%2Ffnut.2024.1474597&partnerID=40&md5=2faf468d3005a2487e6e5acd5bcd4335]
39. Campisi M, Cannella L, Paccagnella O, Brazzale AR, Agnolin A, Grothe T, et al. Unveiling the geroprotective potential of a novel combination of senolytics with natural compounds: A clinical and in vitro study. Biogerontology. 2025;26(2):129-147. doi:10.1007/s10522-024-10167-1.
40. Wang S, Song L, Fan R, Chen Q, Fu R, You M, et al. Nucleotides as an anti-aging supplementation in older adults: A randomized controlled trial (TALENTs study). Advanced science (Weinheim, Baden-Wurttemberg, Germany). 2025 Sep;12(33).
41. Orr ME, Kotkowski E, Ramirez P, Bair-Kelps D, Liu Q, Brenner C, et al. A randomized placebo-controlled trial of nicotinamide riboside in older adults with mild cognitive impairment. GeroScience. 2024 Feb;46(1):665–82.
42. Fuentealba M, Kiprov D, Schneider K, Mu WC, Kumaar PA, Kasler H, et al. Multi-Omics Analysis Reveals Biomarkers That Contribute to Biological Age Rejuvenation in Response to Single-Blinded Randomized Placebo-Controlled Therapeutic Plasma Exchange. Aging Cell. 2025 Aug;24(8):e70103. doi:10.1111/acel.70103.
43. Borsky P, Holmannova D, Parova H, Horvath S, Sramek P, Brooke RT, et al. Human clinical trial of plasmapheresis effects on biomarkers of aging (efficacy and safety trial). Sci Rep. 2025 Jul 1;15(1):21059. doi:10.1038/s41598-025-05396-0.
44. Clement J, Yan Q, Agrawal M, Coronado RE, Sturges JA, Horvath M, et al. Umbilical cord plasma concentrate has beneficial effects on DNA methylation GrimAge and human clinical biomarkers. Aging Cell. 2022 Oct;21(10):e13696. doi:10.1111/acel.13696.
45. Horvath S, Singh K, Raj K, Khairnar S, Sanghavi A, Shrivastava A, et al. Reversal of Biological Age in Multiple Rat Organs by Young Porcine Plasma Fraction. bioRxiv [Preprint]. 2023 Aug 7:2023.08.06.552148. doi:10.1101/2023.08.06.552148.
46. Chiavellini P, Lehmann M, Gallardo MD, Canatelli Mallat M, Pasquini DC, Zoller JA, et al. Young plasma rejuvenates blood DNA methylation profile, extends mean lifespan, and improves physical appearance in old rats. J Gerontol A Biol Sci Med Sci. 2024 May 1;79(5):glae071. doi:10.1093/gerona/glae071.
47. Zhang B, Lee DE, Trapp A, Tyshkovskiy A, Lu AT, Bareja A, et al. Multi-omic rejuvenation and life span extension on exposure to youthful circulation. Nat Aging. 2023 Aug;3(8):948-964. doi:10.1038/s43587-023-00451-9.
48. Horvath S, Lacunza E, Canatelli Mallat M, Portiansky EL, Gallardo MD, Brooke RT, et al. Cognitive rejuvenation in old rats by hippocampal OSKM gene therapy. GeroScience. 2025;47(1):809-823. doi:10.1007/s11357-024-01269-y.