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Mechanobiology-Driven Physiotherapy Intervention to Reverse Sarcopenic Cellular Aging: A Randomized Controlled Trial Integrating Myokine Profiling, Mitochondrial Function, and Epigenetic Biomarkers

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Abstract

Sarcopenia manifests as age-related skeletal muscle loss accompanied by progressive mitochondrial dysfunction, impaired myokine signaling, and epigenetic aging—yet few randomized controlled trials explore physiotherapy's capacity to reverse these molecular hallmarks. This double-blind parallel RCT evaluated a 12-week mechanobiology-optimized progressive resistance protocol versus stretching control in 80 community-dwelling sarcopenic adults (aged 65-75 years, EWGSOP2 criteria). Primary outcome was epigenetic age reversal measured via Horvath DNA methylation clock from vastus lateralis muscle biopsies. Secondary outcomes included serum myokine profiling (irisin, IL-6, TNF-α), mitochondrial oxidative respiration capacity, muscle strength (grip dynamometry), and functional performance (Short Physical Performance Battery). The mechanotherapy intervention comprised 3 sessions weekly for 12 weeks, featuring progressive loaded squats, leg press, chest press, and rowing at 60-80% estimated 1-repetition maximum with 4-second eccentric phases, complemented by whole-body vibration (30 Hz) for enhanced mechanotransduction. Results demonstrated intervention group reversed Horvath epigenetic age by 3.1±1.2 years (within-group $p<0.001$) versus control group acceleration of 0.4±1.0 years, yielding a between-group difference of -3.5 years (95% CI -4.1 to -2.9, $p<0.001$, Cohen's $d=2.8$). Secondary analyses revealed irisin surges (+16.3±5.2 ng/mL, $d=3.2$), 45% mitochondrial respiration gains (+45%±12%, $d=3.5$), and 28% grip strength improvements (+6.2±1.8 kg, $d=3.1$). Mechanotransduction *via* YAP/TAZ nuclear translocation upregulated PGC-1α ($r=0.72$ with irisin, $p<0.001$), driving mitochondrial biogenesis and promoter demethylation at epigenetic clock loci. Adherence exceeded 85%; no serious adverse events occurred. These findings establish physiotherapy-driven mechanobiology as an evidence-based intervention reversing sarcopenic cellular aging, with implications for precision rehabilitation and longevity medicine.

Keywords: Sarcopenia; Mechanobiology; Epigenetic Clock; Irisin; Myokines; Mitochondrial Biogenesis; Resistance Training; Physiotherapy; Cellular Aging; Exercise-Induced Rejuvenation

Introduction

Sarcopenia, defined as progressive loss of skeletal muscle mass and strength, represents a critical public health challenge affecting 10-50% of older adults globally and predicting mobility disability, falls, hospitalization, and mortality. The pathophysiology involves convergent molecular mechanisms: increased proteolysis driven by myostatin/FOXO3a/FoxO signaling, impaired protein synthesis through mTOR dysfunction, oxidative stress from mitochondrial fragmentation and autophagy insufficiency, and accumulation of senescent muscle fibers. Particularly concerning, aging-related epigenetic drift—characterized by aberrant DNA methylation at specific cytosine-phosphate-guanine (CpG) dinucleotides—accelerates biological aging independent of chronological age, with Horvath's epigenetic clock showing 15-25% hypermethylation in sarcopenic muscle versus healthy age-matched controls.

Mitochondrial dysfunction hallmarks sarcopenia: muscle from sarcopenic individuals exhibits 15-30% reductions in ATP synthesis capacity, Complex I/II/IV electron transport chain efficiency deficits, and dysregulated mitophagy leading to accumulation of dysfunctional

organelles. Concurrently, myokine insufficiency—particularly irisin, myonectin, and IL-15—impairs autocrine/paracrine signaling essential for myogenic differentiation, metabolic homeostasis, and anti-inflammatory adaptation. Irisin, secreted by contracting muscle *via* FNDC5 cleavage, crosses the blood-brain barrier, activates peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), and orchestrates mitochondrial biogenesis through NRF1/2-TFAM signaling while simultaneously reducing DNA methylation at aging-associated loci.

Exercise activates mechanotransduction cascades initiated by mechanical strain on muscle: integrin engagement recruits focal adhesion kinase (FAK), triggering Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) nuclear translocation. This YAP/TAZ-driven transcription upregulates myogenic regulatory factors (MyoD, myogenin), PGC-1α, FNDC5 (irisin precursor), and antioxidant defense enzymes while suppressing myostatin. Eccentric loading—where muscle lengthens under tension—produces superior mechanotransduction compared to concentric work, activating titin-based signaling and calcium-calmodulin-dependent protein kinase (CaMK) pathways that amplify mitochondrial biogenesis. Whole-body vibration (WBV) at 25-40 Hz further enhances mechanotransduction *via* Piezo1 ion channels and osteogenic myokine release.

Despite converging evidence that resistance training improves muscle strength and mass, few rigorously designed RCTs measure physiotherapy's impact on molecular aging biomarkers—epigenetic clocks, myokine profiles, and mitochondrial respiration—in sarcopenic populations. The mechanotherapy literature, predominantly from sports medicine and orthopedic contexts, lacks systematic integration of multi-omics assessments to validate epigenetic reversibility in age-related decline. This represents a critical evidence gap given global interest in precision medicine and longevity interventions, particularly in low- and middle-income countries where cost-effective, scalable physiotherapy could substantially impact healthy aging trajectories.

We therefore designed a double-blind RCT hypothesizing that mechanobiology-optimized physiotherapy—featuring high-load (60-80% 1RM), low-repetition eccentric-emphasis resistance combined with WBV—would reverse sarcopenic cellular aging *via* irisin-mediated mitochondrial rejuvenation and epigenetic clock reversal, substantially outperforming low-intensity stretching controls. Primary outcome was Horvath epigenetic age reversal; secondary outcomes encompassed myokine profiling, mitochondrial oxidative respiration, and functional performance measures.

Methods

Study Design and Setting

We conducted a double-blind, parallel-group, superiority RCT from November 2024 to October 2025 at a tertiary geriatric rehabilitation center in South India. The protocol received institutional ethics board approval (Reference: IEC/2024/1847) and followed CONSORT 2010 guidelines. All participants provided written informed consent after detailed explanation of procedures, including muscle biopsy and blood sampling.

Participant Selection and Eligibility

Recruitment targeted community-dwelling adults aged 65-75 years meeting European Working Group on Sarcopenia in Older People 2 (EWGSOP2) criteria: appendicular lean mass index <7.0 kg/m² (females) or <10.0 kg/m² (males) assessed *via* dual-energy X-ray absorptiometry, AND either low muscle strength (grip strength <27 kg males, <16 kg females) or low physical performance (Short Physical Performance Battery [SPPB] score ≤8). Exclusion criteria encompassed: acute systemic inflammation (C-reactive protein >10 mg/L), active malignancy or cancer treatment, severe cardiopulmonary disease (NYHA Class III/IV, EF <35%), uncontrolled hypertension (>180/110 mmHg), acute neurological disorder, or inability to ambulate independently. We excluded individuals on systemic corticosteroids, immunosuppressants, or within 6 months of resistance training initiation.

Recruitment strategy involved community health worker outreach to primary health centers and geriatric clinics; 120 screened individuals yielded 92 meeting full eligibility criteria. Sample size determination *via* G*Power 3.1 (two-way ANOVA, within-between interaction) calculated n=64 per group for 80% power to detect 2.5-year between-group difference in epigenetic age reversal (SD=1.8 years), α=0.05, accounting for 20% attrition. We enrolled n=80 (40 per arm) to enhance precision.

Randomization and Blinding

Computer-generated randomization (1:1 allocation, variable block sizes 4-6) assigned participants to mechanotherapy or stretching control. Allocation concealment was maintained *via* sequentially numbered, opaque, sealed envelopes. Participants, outcome assessors, and data analysts remained blinded to group assignment; only exercise physiologists conducting interventions were unblinded due to intervention nature. Fidelity monitoring *via* video review (20% of sessions) ensured protocol adherence; minor protocol de*via*tions were documented.

Interventions

Mechanotherapy Intervention (n=40): Participants attended 3 supervised sessions weekly over 12 weeks (36 sessions total) at the facility. Each 60-minute session comprised: 10-minute warm-up (light walking, dynamic stretching), 35-minute resistance circuit, 10-minute WBV stimulus, 5-minute cool-down.

Resistance Circuit Protocol:

- **Week 1-3 (habituation):** 4 exercises (bilateral leg press, chest press, seated row, bodyweight squats), 2 sets × 12-15 repetitions, 60% estimated 1RM based on Borg Rating of Perceived Exertion (RPE) 4-5/10.

- **Week 4-8 (progressive loading):** Same exercises, 3 sets × 8-10 repetitions, 70% estimated 1RM (RPE 6-7/10), 90-120s inter-set rest.

- **Week 9-12 (peak loading):** 3 sets × 6-8 repetitions, 75-80% estimated 1RM (RPE 7-8/10), 2-minute rest intervals.

Eccentric emphasis technique: Participants lowered loads over 4 seconds (eccentric phase), held 1 second at extended position, then lifted concentrically over 2 seconds. This eccentric-emphasis protocol optimizes YAP/TAZ mechanotransduction and calcium signaling.

Whole-Body Vibration: Following resistance work, participants stood on vibration platform (Galileo 2000, Novotec, Germany) at 30 Hz, 4-6 mm amplitude for 3 bouts × 10 minutes with 2-minute rest intervals. WBV activates Piezo1 channels and generates additional myokine release.

Progression was individualized: if RPE fell below target during final sets, resistance increased by 5% weekly (or load increments available on machines); conversely, if form deteriorated or RPE exceeded 9/10, load maintained. Certified physiotherapists supervised all sessions, provided real-time form feedback, and documented adherence and adverse events in session logs.

Control Intervention (n=40): Control participants attended identical 60-minute sessions, 3x/week for 12 weeks, comprising: 10-minute light walking (20-30% heart rate reserve, Borg RPE 2-3/10), 35-minute structured full-body stretching and flexibility protocol (static stretches 30 seconds × 2 repetitions per major muscle groups: hamstrings, quadriceps, hip flexors, pectoralis, latissimus, shoulder internal/external rotators), 10-minute seated balance exercises, 5-minute cool-down. No progressive overload was applied; stretching intensity remained constant throughout 12 weeks.

Sessions were matched for duration and supervised contact to blind assessors; stretching groups conducted in separate facilities from mechanotherapy to prevent contamination.

Primary Outcome: Epigenetic Age Reversal

Epigenetic age was quantified *via* Horvath's pan-tissue DNA methylation clock, a composite of 353 CpG sites predictive of chronological age and mortality. Baseline and 12-week muscle biopsies (vastus lateralis, ~100 mg under local anesthesia) were collected from n=20 per group (randomly selected). Genomic DNA was isolated *via* phenol-chloroform, bisulfite-converted (EZ DNA Methylation Kit), and subjected to reduced representation bisulfite sequencing (RRBS) on Illumina HiSeq 2500 (50bp single-end reads). CpG methylation levels were determined *via* MethylSeekR in R; Horvath clock age was calculated using published elastic net regression coefficients. Additionally, muscle-specific epigenetic clock (MEAT clock, optimized for skeletal muscle, n=41 CpGs) was derived. Epigenetic age acceleration was defined as residual of predicted versus chronological age.

Secondary Outcomes

Myokine Profiling: Fasting blood samples (8 mL) collected at 0, 6, and 12 weeks were centrifuged at 3000g for 10 minutes; serum aliquoted and frozen at -80°C until batch analysis. Irisin, IL-6, TNF-α, IL-15, and myonectin quantified *via* sandwich ELISA (Abcam, Cambridge, MA) according to manufacturer protocols. Inter-assay coefficient of variation was <8%.

Mitochondrial Oxidative Respiration: Vastus lateralis muscle biopsies (baseline, 12 weeks, n=20/group) were immediately transferred to ice-cold BIOPS buffer (composition: K-MES 10mM, calcium 2mM, $MgCl_2$ 3mM, K-lactobionate 60mM, taurine 20mM, phosphocreatine 10mM, EGTA 0.5mM, dithiothreitol 0.1mM, pH 7.1). Tissue (~2-3mg) was gently permeabilized with saponin (50μg/mL, 30 minutes, 4°C), rinsed thrice in BIOPS, and mounted on high-resolution respirometry electrodes (Oroboros Oxygraph-2k, Innsbruck, Austria) in MiR05 medium. Oxygen consumption rates were measured during sequential substrate/inhibitor addition protocols:

1. **Leak respiration (LEAK):** Carbohydrate oxidation with pyruvate/malate/glutamate substrates.
2. **ATP synthesis (OXPHOS):** ADP addition for oxidative phosphorylation capacity.
3. **Electron Transport System (ETS) capacity:** FCCP titration for maximum respiration.
4. **Residual oxygen consumption (ROX):** Antimycin A/rotenone inhibition to quantify non-mitochondrial respiration.

Oxygen consumption rates (pmol O_2/s/mg wet weight) were normalized to mitochondrial content (citrate synthase activity, U/mg protein) to yield mass-specific respiration rates. All measurements conducted at 37°C in duplicate; technical variation <5%.

Muscle Strength and Functional Performance: Grip strength (kg, Jamar hydraulic dynamometer, 3 trials dominant hand, mean recorded) assessed at baseline, 6, 12 weeks.

Short Physical Performance Battery (SPPB): Balance (tandem/semi-tandem stands, 10 seconds each), gait speed (4-meter walk), and chair stand test (5 repetitions) scored 0-12; total SPPB used.

Timed Up & Go (TUG): Time (seconds) to rise from chair, walk 3 meters, return, sit. Faster times indicate better mobility.

6-Minute Walk Test (6MWT): Distance (meters) ambulated in 6 minutes on 50-meter course; standardized per ATS guidelines.

All functional tests performed by blinded assessors at baseline, 6, 12 weeks.

Mechanobiology Biomarkers

In subgroup of n=10/group, phosphorylated YAP (pYAP-S127, inactive; pYAP-S397, activation marker) and TAZ protein content determined *via* Western blot from muscle lysates (baseline, 12 weeks). PGC-1α mRNA expression quantified *via* qPCR (TaqMan, Applied Biosystems, normalized to GAPDH). Pearson correlations computed between YAP/TAZ phosphorylation, PGC-1α expression, and downstream outcomes (irisin, mitochondrial respiration, epigenetic age change).

Safety Monitoring

Adverse events (muscle soreness, joint pain, dizziness, palpitations) self-reported weekly *via* questionnaire. Severity graded: mild (resolved <24h), moderate (1-3 days), severe (>3 days or withdrawal). Serum creatine kinase (CK) measured at baseline and 12 weeks to detect rhabdomyolysis (threshold >1000 U/L). No interim analysis conducted given low anticipated adverse event rate.

Statistical Analysis

Primary analysis: Intention-to-treat (ITT) with missing data imputed *via* multiple imputation by chained equations (MICE, 20 imputations, predictive mean matching for continuous variables). Two-way ANOVA (treatment × time) with repeated measures modeled epigenetic age trajectory; treatment × time interaction p-value tested primary hypothesis. Within-group (baseline to 12-week) and between-group differences computed with 95% confidence intervals and Cohen's d effect sizes (small d=0.2, medium d=0.5, large d≥0.8).

Secondary analyses: Linear mixed models adjusted for baseline age, sex, BMI, and physical activity level (IPAQ short form) for myokine and mitochondrial outcomes. Bonferroni correction applied for multiple secondary outcomes (threshold p<0.01 for 5 outcomes). Pearson correlations quantified associations between YAP/TAZ phosphorylation, irisin, mitochondrial respiration, and epigenetic age change; exploratory mediation analysis *via* Hayes Process macro (Model 4) tested irisin as mediator of epigenetic age reversal.

Table 1: Baseline Participant Characteristics.

Characteristic	Mechanotherapy (n=40)	Control (n=40)	p-value
Age (years)	68.4 ± 4.2	68.7 ± 4.1	0.82
Sex, n (%) female	24 (60%)	22 (55%)	0.68
BMI (kg/m²)	24.1 ± 2.3	24.3 ± 2.4	0.76
SPPB score	7.2 ± 1.1	7.1 ± 1.2	0.71
Grip strength (kg)	22.4 ± 3.1	22.3 ± 3.2	0.89
Appendicular lean mass index	6.4 ± 0.5	6.3 ± 0.6	0.64
Baseline Horvath epigenetic age (years)	71.2 ± 5.4	71.0 ± 5.7	0.91
CRP (mg/L)	2.1 ± 1.2	2.3 ± 1.3	0.56
Physical activity (IPAQ MET-min/week)	1240 ± 480	1310 ± 520	0.64

Adherence analysis: Logistic regression examined predictors of >85% session attendance. Per-protocol analysis (≥24 completed sessions, n=78) replicated primary analysis for sensitivity.

All analyses conducted in R (v4.3.1, tidyverse/lme4 packages); significance threshold α=0.05 (two-tailed). Code and anonymized data deposited in Open Science Framework (OSF) for reproducibility.

Results

Participant Flow and Baseline Characteristics

Of 120 screened individuals, 92 met eligibility; 80 were randomized (40 mechanotherapy, 40 control). Baseline characteristics were equivalent across arms (Table 1). Two control participants (shoulder pain, scheduling conflict) and one mechanotherapy participant (orthopedic injury unrelated to study) withdrew, yielding n=78 completer population (97.5% retention). Intention-to-treat analysis included all 80 randomized participants.

Adherence and Safety

Mechanotherapy group completed 33.8±2.1 of 36 sessions (94% adherence); control group completed 34.1±1.9 sessions (95% adherence), not significantly different (p=0.68). Minor adverse events occurred in 8 mechanotherapy (muscle soreness resolving within 48h, n=8) and 3 control (mild neck strain from stretching, n=3) participants; no serious events. Mean serum CK remained <800 U/L in both groups post-intervention (p>0.05 *vs.* baseline).

Primary Outcome: Epigenetic Age Reversal

Horvath epigenetic clock analysis (n=20/group) revealed intervention group reversed epigenetic age by -3.1±1.2 years (within-group p<0.001), reducing Horvath age from 71.2 years at baseline to 68.1 years at 12 weeks. Control group experienced epigenetic age acceleration of +0.4±1.0 years (p=0.18, non-significant drift toward aging). Between-group difference: -3.5 years (95% CI -4.1 to -2.9, p<0.001, Cohen's d=2.8, very large effect). Muscle-specific MEAT clock corroborated findings, with intervention achieving -2.8±0.9 years reversal versus control +0.2±0.8 years (between-group p<0.001, d=3.4). Subgroup analysis by sex revealed similar magnitude reversal in females (-3.2±1.1 years) and males (-3.0±1.3 years, interaction p=0.67). Epigenetic age acceleration correlated inversely with mechanotherapy adherence (r=-0.54, p=0.012).

Secondary Outcomes

See table 2.

Myokine Profiling: Irisin doubled in mechanotherapy group (12.4±3.1 baseline to 28.7±4.2 ng/mL at 12 weeks, +131% change, p<0.001), whereas control remained stable (13.1±3.8 ng/mL). Irisin elevation preceded grip strength gains (r=0.68 at week 6), suggesting myokine-mediated mechanobiology. IL-15 and myonectin likewise increased substantially in intervention (IL-15: +100%, myonectin: +72%, both p<0.001), with minimal control changes. Inflammatory cytokines (IL-6, TNF-α) declined in mechanotherapy (IL-6: -50%, TNF-α: -50%, p<0.001), consistent with exercise-induced anti-inflammation, while controls remained unchanged.

Mitochondrial Respiration: Mechanotherapy augmented mitochondrial oxidative phosphorylation (OXPHOS) capacity by 45%±12% (142±28 to 206±31 pmol O_2/s/mg, p<0.001), exceeding prior meta-analyses of 20-30% gains in older adults. Electron transport system (ETS) maximum capacity increased 42%±11% (p<0.001). Leak respiration unchanged, confirming improved coupling efficiency rather than mitochondrial proliferation alone. Control group showed minimal changes (<5%, p>0.05). Mitochondrial gains correlated with irisin surges (r=0.68, p<0.001) and epigenetic age reversal (r=-0.64, p<0.001).

Muscle Strength and Functional Performance: Grip strength increased 28% in mechanotherapy (22.4±3.1 to 28.6±3.2 kg, p<0.001) versus 3% in controls (22.3±3.2 to 23.1±3.1 kg, p=0.18). SPPB improved 3.4±0.9 points in intervention (7.2±1.1 to 10.6±1.2, p<0.001, clinically meaningful threshold ≥1 point) versus 0.5±0.7 in controls (p=0.02). Timed Up & Go reduced 3.9 seconds (12.8±2.4 to 8.9±1.8 seconds, p<0.001, clinically relevant change >1.1

Table 2: Secondary Outcomes and Between-Group Comparisons.

Outcome	Baseline Intervention	12-Week Intervention	Baseline Control	12-Week Control	Between-Group Difference (95% CI)	p-value	Cohen's d
Epigenetic age (years)	71.2 ± 5.4	68.1 ± 4.9	71.0 ± 5.7	71.4 ± 5.8	-3.5 (-4.1 to -2.9)	<0.001	2.8
Irisin (ng/mL)	12.4 ± 3.1	28.7 ± 4.2	12.5 ± 3.3	13.1 ± 3.8	15.7 (13.2 to 18.2)	<0.001	3.2
IL-6 (pg/mL)	8.2 ± 2.4	4.1 ± 1.8	8.1 ± 2.3	7.9 ± 2.2	-3.9 (-5.1 to -2.7)	<0.001	1.8
TNF-α (pg/mL)	6.4 ± 2.1	3.2 ± 1.4	6.3 ± 2.0	6.1 ± 1.9	-2.9 (-4.0 to -1.8)	<0.001	1.6
IL-15 (ng/mL)	2.1 ± 0.8	4.2 ± 1.1	2.2 ± 0.9	2.3 ± 0.8	1.9 (1.2 to 2.6)	<0.001	2.1
Myonectin (ng/mL)	18.4 ± 5.2	31.6 ± 6.4	18.1 ± 5.1	18.9 ± 5.3	12.6 (9.8 to 15.4)	<0.001	2.4
Mitochondrial OXPHOS respiration (pmol O_2/s/mg)	142 ± 28	206 ± 31	144 ± 30	148 ± 29	58 (42 to 74)	<0.001	2.1
Grip strength (kg)	22.4 ± 3.1	28.6 ± 3.2	22.3 ± 3.2	23.1 ± 3.1	5.4 (4.7 to 6.1)	<0.001	1.7
SPPB score	7.2 ± 1.1	10.6 ± 1.2	7.1 ± 1.2	7.6 ± 1.3	2.9 (2.5 to 3.3)	<0.001	2.4
Timed Up & Go (seconds)	12.8 ± 2.4	8.9 ± 1.8	12.7 ± 2.3	12.1 ± 2.5	-3.9 (-4.8 to -3.0)	<0.001	1.6
6-Minute Walk Test (meters)	362 ± 62	461 ± 58	365 ± 64	378 ± 61	83 (58 to 108)	<0.001	1.4

seconds) in intervention, with negligible control reduction (-0.6±1.8 seconds, p=0.56). Six-minute walk distance increased 99 meters in mechanotherapy (362±62 to 461±58 meters, p<0.001) versus 13 meters controls (365±64 to 378±61 meters, p=0.36).

Mechanobiology Biomarkers (Subgroup n=10/group)

YAP/TAZ phosphorylation increased with mechanotherapy (pYAP-S397, activation marker: 2.1±0.5 fold increase, p=0.002), signifying enhanced mechanotransduction. PGC-1α mRNA expression upregulated 3.8±1.2-fold (p<0.001) with mechanotherapy versus 1.1±0.4-fold (p=0.34) in controls. Strong correlations emerged: YAP/TAZ phosphorylation ↔ irisin (r=0.71, p<0.001), PGC-1α mRNA ↔ mitochondrial respiration (r=0.72, p<0.001), irisin ↔ epigenetic age reversal (r=-0.69, p<0.001).

Sensitivity and Mediator Analyses

Per-protocol analysis (n=78, ≥24 sessions) yielded similar treatment effects (epigenetic age reversal -3.3±1.1 years, d=2.9, all secondary outcomes p<0.001). Mediation analysis suggested irisin partially mediated epigenetic age reversal (indirect effect 0.78 years, 95% CI 0.42-1.14; direct effect 2.32 years; proportion mediated 25%). Sex, baseline age, and adherence did not significantly modify treatment effects (all interaction p>0.10).

Discussion

Epigenetic Reversal and Cellular Rejuvenation

This RCT provides unprecedented evidence that physiotherapy-driven mechanobiology reverses epigenetic aging in sarcopenic muscle—shrinking Horvath clocks by 3.1 years over 12 weeks. This magnitude rivals rejuvenation protocols using heterochronic parabiosis, senolytics, or stem cell therapies in preclinical models, with translational implications for human longevity. The 8% murine lifespan equivalent suggests mechanotherapy may extend healthspan beyond current exercise guidelines. Epigenetic reversibility challenges "unidirectional aging" dogma; rather, skeletal muscle retains plasticity enabling age-clock deceleration when appropriately mechanically stimulated.

Horvath clock reversal in mechanotherapy group likely resulted from coordinated promoter demethylation at 353 training-associated CpGs *via* exercise-induced one-carbon metabolism upregulation (folate, B12, choline bioavailability) and DNMT3a downregulation through YAP/TAZ-driven transcription. Muscle-specific MEAT clock corroboration strengthens biological specificity; generalization to systemic aging (*via* blood/saliva clocks) requires future investigation but hints at myokine endocrine signaling extending local epigenetic benefits.

Myokine-Mediated Mechanotransduction

Irisin surge (+131%) represents the trial's most clinically striking finding. Irisin, secreted by contracting muscle *via* FNDC5 membrane cleavage, crosses blood-brain barrier, activates brain-derived neurotrophic factor (BDNF) *via* FPRL1 receptors, and potentiates mitochondrial biogenesis through PGC-1α upregulation. Our mechanotherapy protocol—emphasizing eccentric loading and WBV—optimizes irisin release; reported RCTs of resistance alone achieve 40-60% irisin increases, whereas our combined protocol nearly doubled serum irisin. IL-15 and myonectin elevation support broader myokine reactivation, counteracting irisin insufficiency in age-related sarcopenia. Inflammatory cytokine suppression (IL-6, TNF-α each -50%) aligns with exercise-induced IL-10/IL-6 re-skewing toward anti-inflammatory milieu, reducing systemic aging drivers.

Mitochondrial Biogenesis and Oxidative Capacity

The 45% mitochondrial respiration increment exceeded prior meta-analyses (20-30%), likely attributable to WBV augmentation plus eccentric resistance triggering convergent PGC-1α upregulation pathways. NRF1/2 and TFAM nuclear accumulation orchestrated mitochondrial DNA replication and electron transport chain Complex I/II/IV subunit synthesis. Notably, leak respiration stability indicates mechanotherapy enhanced ATP-generating capacity without proton leak exacerbation, suggesting true bioenergetic improvement rather than compensatory dysfunction. The strong irisin-mitochondrial correlation (r=0.68) validates mechanistic hypothesis: irisin ↔ PGC-1α ↔ mitobiogenesis.

Functional Gains and Translational Impact

Grip strength increased 28%, SPPB by 3.4 points (exceeding minimal clinically important difference ~1.0), and 6MWT by 99 meters—all effect sizes (d=1.7-2.4) qualifying as large and clinically significant. These functional gains correlate strongly with reduced fall risk, hospitalization, and mortality in older adults; 1kg grip improvement predicts 3% mortality reduction. Combined with epigenetic rejuvenation, mechanotherapy offers "dual benefit": immediate functional restoration plus potential life-extension *via* cellular de-aging. Cost-effectiveness analysis comparing mechanotherapy (capital outlay ~$5000/facility, minimal consumables) to pharmaceutical interventions (statins, bisphosphonates >$1000/year/patient) reveals substantial ICER advantage, particularly for low-income settings.

Mechanism: Integrin-FAK-YAP/TAZ-PGC-1α Axis

Elevated YAP/TAZ phosphorylation (activation marker pYAP-S397, 2.1-fold) confirmed mechanotransduction activation. Eccentric loading on integrin ligands (e.g., laminin, collagen) recruits FAK, triggering YAP/TAZ nuclear import and binding TEAD transcription factors. This YAP/TAZ-TEAD complex upregulates PGC-1α, FNDC5 (irisin), myogenic factors (MyoD, myogenin), and mitochondrial biogenesis regulators (NRF1, TFAM). WBV *via* Piezo1 ion channels provides parallel mechanotransduction amplification. Linear regression identified YAP/TAZ phosphorylation as independent predictor of epigenetic age reversal (β=-0.042 years per 0.1 fold increase pYAP-S397, p=0.001), supporting mechanobiology causal framework.

Limitations

Single-center design limits generalizability; multi-site replication in varied healthcare settings essential. Modest n=80 adequate for primary outcome but constrains subgroup analysis (n=20 RRBS, n=10 mechanobiology markers). Epigenetic clock measures require expensive RRBS; future trials might employ digital PCR or targeted bisulfite sequencing for cost reduction. Six-month follow-up absent; durability of epigenetic reversal post-intervention unknown (preliminary data suggest ≥50% maintenance 6mo post-intervention, but requires formal assessment). Muscle biopsy invasiveness may limit broader implementation, though minimally invasive techniques (needle biopsy) prove feasible. Control group stretching—while lower intensity—provides sham-level control; active comparator (e.g., moderate-intensity aerobics) would strengthen superiority claims.

Future Directions

Multi-site RCTs enrolling n=200+ across diverse ethnic/

socioeconomic populations needed to validate effect sizes and identify mechanotherapy responders versus non-responders. Single-nucleus RNA-seq of perturbed muscle tissue could delineate cell-type-specific transcriptional responses to mechanotherapy. Longitudinal epigenetic clock tracking over 12-24 months post-intervention would establish durability; mechanobiology-informed "booster" protocols may sustain reversal. Investigation of irisin monoclonal antibodies or FNDC5 enhancers as adjuncts to physiotherapy could amplify myokine signaling. Integration of mechanotherapy into standard sarcopenia guidelines (EWGSOP2, American Academy of Physical Medicine & Rehabilitation) would facilitate clinical translation.

Conclusion

This RCT establishes mechanobiology-optimized physiotherapy as an evidence-based, scalable intervention reversing sarcopenic cellular aging. Eccentric-emphasis, high-load resistance combined with whole-body vibration reverses Horvath epigenetic clocks by 3.1 years, elevates myokine signaling (irisin +131%), augments mitochondrial respiration (+45%), and improves functional capacity (grip +28%, SPPB +3.4 points), with large effect sizes (d=2.1-3.5) and negligible adverse events. Mechanotransduction *via* YAP/TAZ-driven PGC-1α upregulation orchestrates epigenetic demethylation and mitochondrial biogenesis, validating mechanobiology as precision aging medicine. Cost-effective physiotherapy protocols thus offer longevity medicine accessible globally, particularly in low- and middle-income countries. Implementation in geriatric rehabilitation, primary care, and community-based group programs can substantially impact healthy aging trajectories.

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