SUPPLEMENTARY DATA

Aging Biomarkers in Osteoporosis: A Systematic Review of Molecular Mechanisms, Biological Aging, and Population Evidence

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Supplementary Table 1. PRISMA 2020 for Abstracts Checklist and PRISMA 2020 Checklist

PRISMA 2020 for Abstracts Checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Y
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Y
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Y
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Y
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Y
Synthesis of results	6	Specify the methods used to present and synthesise results.	Y
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Y
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Y
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Y
Interpretation	10	Provide a general interpretation of the results and important implications.	Y
OTHER			
Funding	11	Specify the primary source of funding for the review.	N/A
Registration	12	Provide the register name and registration number.	N/A

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	1-3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	5-8
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	5-6, Supplementary Table 2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	6-7
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	7
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	7
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	7
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	8
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	7
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	6-7
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	7
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	N/A
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	N/A
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	8

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Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Figure 1, 2
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Supplementary Table 3, 4
Study characteristics	17	Cite each included study and present its characteristics.	Table 1, 2
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Supplementary Table 5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Table 1, 2
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	9, 11
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	9-13
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	9-13
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	N/A
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	N/A
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	10-11, 13
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	13
	23b	Discuss any limitations of the evidence included in the review.	13-14
	23c	Discuss any limitations of the review processes used.	14
	23d	Discuss implications of the results for practice, policy, and future research.	14-15
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	9
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	9
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	N/A
Competing interests	26	Declare any competing interests of review authors.	N/A
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	15

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Supplementary Table 2. Database Search Queries for Selection Process

	PubMed	Embase
Epigenetic Aging Clocks	(("epigenetic"[Title/Abstract] AND "aging"[Title/Abstract]) OR "epigenetic age"[Title/Abstract] OR "DNAm age"[Title/Abstract] OR "DNA methylation age"[Title/Abstract] OR "epigenetic clock"[Title/Abstract] OR "Hannum"[Title/Abstract] OR "Horvath"[Title/Abstract] OR "GrimAge"[Title/Abstract] OR "PhenoAge"[Title/Abstract]) AND ("osteoporosis"[Title/Abstract] OR "BMD"[Title/Abstract] OR "fracture"[Title/Abstract] OR "bone loss"[Title/Abstract] OR "bone mineral density"[Title/Abstract]) NOT (review[pt] OR systematic[sb] OR congress[pt]) AND ("2013"[Date - Publication] : "3000"[Date - Publication])	('epigenetic':ti,ab,kw AND aging:ti,ab,kw OR 'epigenetic age':ti,ab,kw OR 'dnam age':ti,ab,kw OR 'dna methylation age':ti,ab,kw OR 'epigenetic clock':ti,ab,kw OR hannum:ti,ab,kw OR horvath:ti,ab,kw OR grimage:ti,ab,kw OR phenoage:ti,ab,kw) AND (osteoporosis:ti,ab,kw OR bmd:ti,ab,kw OR fracture:ti,ab,kw OR 'bone loss':ti,ab,kw OR 'bone mineral density':ti,ab,kw) AND [2013-2025]/py NOT ('review'/exp OR 'systematic review'/exp OR 'conference abstract'/exp OR 'conference paper'/exp)
Leukocyte Telomere Length	("leukocyte telomere length"[Title/Abstract] OR "telomere length"[Title/Abstract] OR "LTL"[Title/Abstract]) AND ("bone mineral density"[Title/Abstract] OR "osteoporosis"[Title/Abstract] OR "BMD"[Title/Abstract] OR "bone loss"[Title/Abstract] OR "fracture"[Title/Abstract]) NOT (review[pt] OR systematic[sb] OR congress[pt]) AND ("2002"[Date - Publication] : "3000"[Date - Publication])	('leukocyte telomere length':ti,ab,kw OR 'telomere length':ti,ab,kw OR LTL:ti,ab,kw) AND ('bone mineral density':ti,ab,kw OR osteoporosis:ti,ab,kw OR BMD:ti,ab,kw OR 'bone loss':ti,ab,kw OR fracture:ti,ab,kw) AND [2002-2025]/py NOT ('review'/exp OR 'systematic review'/exp OR 'conference abstract'/exp OR 'conference paper'/exp)

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Supplementary Table 3. Reasons for Exclusions after Full-text Assessment for Epigenetic Aging-related Literature

Literature	*Reason for Exclusion*
Jintaridth, 2013	Examined global methylation of Alu repetitive elements, not epigenetic aging clocks.
Riancho, 2015	Commentary, no mentions of epigenetic aging clocks
Del Real, 2017	Analyzed methylation signatures in mesenchymal stem cells rather than blood-based epigenetic aging clocks. Did not check associations between epigenetic aging measures and osteoporosis-related outcomes.
Simpkin, 2017	Primarily investigates child and adolescent development instead of adult bone outcomes.
Zhang, 2024	Review article, no original data
Tarpada, 2025	Research outcome defined as 1-year mortality following hip fracture, which is not one of our primary osteoporosis-related outcomes.

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Supplementary Table 4. Reasons for Exclusions after Full-text Assessment for Telomere Length-related Literature

Literature	*Reason for Exclusion*
Price, 2002	Telomere length measured through TRF in osteoarthritic cartilage chondrocytes. No required osteoporosis-related outcomes.
Bekaert, 2005	Telomere length measured through TRF.
Valdes, 2007	Telomere length measured through TRF.
Harley, 2013	Primary exposure was a telomerase-activating supplement instead of telomere length. BMD was evaluated as a treatment response.
Ribero, 2015	Primary exposure was naevus count. Telomere length evaluated as covariate.
Woo, 2016	Primary exposure is walking speed. Primary outcome is frailty. No examination of osteoporosis-related outcomes.
Kalyan, 2017	Telomere length was not the primary exposure. Association with bone outcomes not evaluated.
Lee, 2021	Local tissue TL and osteonecrosis outcome. No blood TL or osteoporosis-related outcomes.
Hong, 2023	Primarily investigates children instead of adult bone outcomes. Bone mass as exposure and LTL as outcome.
Yeung, 2023	Did not include any osteoporosis-related outcomes.
Du, 2024	Outcomes were serum bone metabolism markers, which are not our primary osteoporosis-related outcomes.
Shiau, 2024	Explored epigenetic aging markers and DNAmTL instead of LTL measurements.
Schoeler, 2025	Examined telomere length and bone mineral density as MR risk factors for cognitive and physical decline, instead of evaluating association between telomere length and osteoporosis-related outcomes.

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Supplementary Table 5. Risk of Bias Assessment for Epigenetic Aging-related Studies

Case-control, cohort studies				
	Selection (4)	Comparability (2)	Outcome (3)	Total (9)
Fernandez-Rebollo, 2018	★		★★★	★★★★
Wei, 2024 (cohort portion)	★★★★	★★	★★★	★★★★★★★★★
Cross-sectional studies				
	Selection (5)	Comparability (2)	Outcome (3)	Total (10)
Shiau, 2024	★★★		★★★	★★★★★★
Wei, 2024 (cross-sectional portion)	★★★★	★★	★★★	★★★★★★★★★
Zhu, 2024	★★★★	★	★★★	★★★★★★★★★
Mendelian randomization studies				
Liang, 2023	**Core assumptions:** Genetic instruments were strong and pleiotropy was formally assessed, but limited SNP numbers mean residual pleiotropy cannot be fully excluded. **Methods reporting:** This was a clearly reported assumed independent two-sample MR using European GWAS data with LD-pruned instruments. **Data presentation:** Instrumental variable estimates and multiple sensitivity analyses were presented transparently. Results were not compared with observational estimates. **Interpretation:** Most findings were null or method-inconsistent, with wide confidence intervals limiting interpretability. **Clinical implications:** Evidence is insufficient to guide clinical practice.			

Case-control, cohort, cross-sectional studies: Newcastle–Ottawa Scale. An adjusted version was used for cross-sectional studies.
Mendelian randomization studies: Davies et al. 2018 Box 2. Critical appraisal checklist for evaluating Mendelian randomisation studies.

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Supplementary Table 6. Risk of Bias Assessment for Telomere-related Studies

Case-control, cohort studies				
	Selection (4)	Comparability (2)	Outcome (3)	Total (9)
Sanders, 2009 (cohort portion)	★★★	★★	★★	★★★★★★★
Tang, 2010 (cohort portion)	★★★	★★	★★★	★★★★★★★★
Curtis, 2022	★★★	★★	★★★	★★★★★★★★
Kirk, 2022	★★★★	★★	★★★	★★★★★★★★★
Han, 2024	★★★★	★★	★★★	★★★★★★★★★
Kirk, 2024	★★★★	★★	★★★	★★★★★★★★★
Di, 2025	★★★★	★★	★★★	★★★★★★★★★
Cross-sectional studies				
	Selection (5)	Comparability (2)	Outcome (3)	Total (10)
Sanders, 2009 (cross-sectional portion)	★★★	★★	★★★	★★★★★★★★
Tang, 2010 (cross-sectional portion)	★★	★★	★★★	★★★★★★★
Tamayo, 2010		★	★★★	★★★★
Nielsen, 2015	★★	★	★★★	★★★★★★
Kalyan, 2018	★★★	★★	★★★	★★★★★★★★
Tao, 2019	★★★	★★	★★★	★★★★★★★★
Guo, 2023	★★★★	★★	★★★	★★★★★★★★★
Mendelian randomization studies				
Wu, 2021	**Core assumptions:** Strong instruments were used (F = 24.19), confounder-associated SNPs were excluded, and pleiotropy was assessed, though residual pleiotropy cannot be fully excluded. **Methods reporting:** Assumed independent two-sample MR using predominantly European GWAS summary data, with LD-pruned independent SNPs and allele harmonization. **Data presentation:** Results were presented as instrumental variable estimates with extensive sensitivity analyses, not compared with observational estimates. Full SNP and GWAS data were provided for reproducibility. **Interpretation:** Most findings were null and method-consistent, suggesting no causal effect of leukocyte telomere length on bone mineral density, though with wide confidence intervals. **Clinical implications:** Evidence is insufficient to guide clinical practice.			

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Sun, 2024	**Core assumptions:** Genetic variants strongly associated with LTL and SHBG, confounder associations screened, and horizontal pleiotropy assessed, though residual pleiotropy cannot be fully excluded. **Methods reporting:** Assumed independent two-sample MR using predominantly European GWAS summary data, with LD-pruned independent SNPs and allele harmonization. **Data presentation:** Results were presented as instrumental variable estimates with extensive sensitivity analyses, not compared with observational estimates. Full SNP and GWAS data were provided for reproducibility. **Interpretation:** Univariable MR suggested a protective effect of longer LTL on osteoporosis while multivariate MR not, with wide confidence intervals, limiting interpretability. **Clinical implications:** Evidence is insufficient to guide clinical practice.

Case-control, cohort, cross-sectional studies: Newcastle–Ottawa Scale. An adjusted version was used for cross-sectional studies.

Mendelian randomization studies: Davies et al. 2018 Box 2. Critical appraisal checklist for evaluating Mendelian randomisation studies.