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Review article

Measuring biological age: Insights from omics studies

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Abbreviations: 5-mCpG, CpG Methylation; βHB, β-hydroxybutyrate; ACP1, Acid Phosphatase 1; ANKRD20A9P, Ankyrin Repeat Domain 20 Family Member A9, Pseudogene; AI, Artificial Intelligence; AKG, α-ketoglutarate; AMED, Alternative Mediterranean Diets; APOE, Apolipoprotein E; ARFIP1, ARF Interacting Protein 1; ASC, Apoptosis-Associated Speck-Like Protein Containing a CARD; ASGR1, Asialoglycoprotein Receptor 1; ATXN2L, Ataxin 2 Like; BAA, Biological Age Acceleration; BMI, Body Mass Index; C9, complement component C9; CAC, Coronary Artery Calcium; CD46, CD46 Molecule; CDCP1, CUB domain-containing protein 1; CDKN2A, Cyclin Dependent Kinase Inhibitor 2 A; CDKN2B, Cyclin Dependent Kinase Inhibitor 2B; CELSR2, Cadherin EGF LAG Seven-Pass G-Type Receptor 2; CEP170, Centrosomal Protein 170; CNNs, Convolutional Neural Networks; COA1, Cytochrome C Oxidase Assembly Factor 1; COMMD1, Copper Metabolism Domain Containing 1; CRF, Cardiorespiratory Fitness; CRP, C-Reactive Protein; CVD, Cardiovascular Diseases; CXCL8, C-X-C Motif Chemokine Ligand 8; CXCL12, C-X-C Motif Chemokine Ligand 12; CXCL13, C-X-C Motif Chemokine Ligand 13; DASH, Dietary Approaches to Stop Hypertension; DHA, Docosahexaenoic Acid; DI-GM, Dietary Index for Gut Microbiota; DNAmTL, DNA methylation derived Telomere Length; DNN, Deep Neural Network; DPY30, Dpy-30 Histone Methyltransferase Complex Regulatory Subunit; EAA, Epigenetic Age Acceleration; EFEMP1, EGF-Like Fibulin Extracellular Matrix Protein 1; EGCG, Epigallocatechin-3-gallate; EPA, Eicosapentaenoic Acid; EWAS, Epigenome-Wide Association Studies; FOXO3, Forkhead Box O3; FURIN, Furin, Paired Basic Amino Acid Cleaving Enzyme; FVC, Forced Vital Capacity; GA, Gestational Age; GAA, Gestational Age Acceleration; GAS6, Growth Arrest Specific 6; GDM, Gestational Diabetes Mellitus; GEMs, Genome-Scale Metabolic models; GNHS, Guangzhou Nutrition and Health Study; GPR78, G Protein-Coupled Receptor 78; GWAS, Genome-Wide Association Studies; HDAC, Histone Deacetylases; HEXIM1, HEXIM P-TEFb Complex Subunit 1; HIIT, High Intensity Intermittent Training; HLA-E, Major Histocompatibility Complex, Class I, E; HPDI, Healthy PDI; HTS, High-Throughput Sequencing; IGF2, Insulin-Like Growth Factor 2; IGFBP4, Insulin Like Growth Factor Binding Protein 4; IL1RN, Interleukin 1 Receptor Antagonist; IL-6, Interleukin 6; IL-β, Interleukinβ; InDels, Insertions/Deletions; KDM, Klemera-Doubal Method; KIT, KIT Proto-Oncogene, Receptor Tyrosine Kinase; KYNA, Kynurenic Acid; LDSC, Linkage Disequilibrium Score Regression; LHFPL6, LHFPL Tetraspan Subfamily Member 6; LINC00202 FAM238C, Family With Sequence Similarity 238 Member C; LPL, Lipoprotein Lipase; LRG1, leucine-rich alpha-2-glycoprotein; LTL, Leucocyte Telomere Length; MAX, MYC Associated Transcriptional Regulator X; MedDiet, Mediterranean Diet; MIND, Mediterranean-DASH Intervention for Neurodegenerative Delay; MMP9, matrix metallopeptidase 9; MPO, myeloperoxidase; MR, Mendelian Randomization; MSR1, Macrophage scavenger receptor types I and II; MTA, Metabolic Transformation Algorithm; MtDNA, Mitochondrial DNA; NAC, N-acetylcysteine; NAD⁺, Nicotinamide adenine dinucleotide; NECS, New England Centenarian Study; NESDA, Netherlands Study of Depression and Anxiety; NFATC1, Nuclear Factor Of Activated T Cells 1; NGS, Next-Generation Sequencing; NMN, Nicotinamide Mononucleotide; NMR, Nuclear Magnetic Resonance; NR, Nicotinamide Riboside; NR/NMN, Nicotinamide Riboside/Nicotinamide Mononucleotide; NT5C3A, 5''-Nucleotidase, Cytosolic IIIA; ONT, Oxford Nanopore Technologies; PARP1, PolyADP-Ribose Polymerase 1; PC, Principal Components; PDI, Plant-Based Diet Index; PGC-1α, Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha; PHB1, Prohibitin 1; PPARγ, , Peroxisome Proliferator-Activated Receptor Gamma); PRS, Polygenic Risk Score; PSMB1, Proteasome 20S Subunit Beta 1; PQTL, Protein Quantitative Trait Loci; RALY, RALY Heterogeneous Nuclear Ribonucleoprotein; RNNs, Recurrent Neural Networks; ROS, Reactive Oxygen Apecies; RPA2, Replication Protein A2; RTC, Randomized Controlled Trial; S100-A8, S100 Calcium Binding Protein A8; S100-A9, S100 Calcium Binding Protein A9; SAM, S-adenosylmethionine; SDCCAG8, SHH Signaling And Ciliogenesis Regulator SDCCAG8; SERPINA1, Serpin Family A Member 1; SERPINF2, Serpin Family F Member 2; SHISA5, Shisa Family Member 5; SIT, Sprint Interval Training; SMRT, Single-Molecule Real-Time Sequencing; SNP, Single Nucleotide Polymorphisms; SPRED2, Sprouty Related EVH1 Domain Containing 2; STK17A, Serine/Threonine Kinase 17a; PTPRS, receptor-type tyrosine-protein phosphatase S; STRs, Short Tandem Repeats; SVM, Support Vector Machines; SVR, Support Vector Regression; TCA, Tricarboxylic Acid; TK1, Thymidine Kinase 1; TL, Telomere Length; TNFα, Tumor Necrosis Factor α; TRAPPC3, Trafficking protein particle complex subunit 3; TRDMT1, TRNA Aspartic Acid Methyltransferase 1; TYMP, Thymidine Phosphorylase; UMOD, Uromodulin; UPDI, Unhealthy PDI; VEGFA, Vascular Endothelial Growth Factor A; XAI, explainable/interpretable AI; WFDC2, WAP four-disulphide core domain protein 2; WGBS, Whole Genome Bisulfite Sequencing; YTHDC1, YTH N6-Methyladenosine RNA Binding Protein C1; ZPR1, ZPR1 Zinc Finger.

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ABSTRACT

Biological ageing is a systemic, multifactorial process driven by progressive molecular and cellular alterations whose complexity necessitates systems-level approaches. Advances in high-throughput omics technologies now allow simultaneous quantification of millions of biomolecules from a single specimen, enabling longitudinal, integrative profiling across multiple molecular layers. This review synthesizes recent progress in applying genomics, epigenomics, metabolomics and microbiomics to ageing research, highlighting their contributions to biomarker discovery, mechanistic insight, and translational opportunities. Genomic studies reveal genetic variants that promote extreme longevity, while epigenetic clocks provide robust predictors of biological age. The blood proteome can be used to calculate proteome-based scores and evaluate temporal changes in ageing trajectories in an organ- and sex-specific manner. Metabolomic signatures identify key metabolites reflecting ageing trajectories, and microbiome research demonstrates that gut microbial composition mirrors and modulates biological ageing, with microbiome clocks emerging. The omics approaches have further elucidated the impact of exercise and diet providing evidence that interventions can reduce biological age. The integration of multi-omics with clinical and lifestyle data, powered by machine learning and artificial intelligence, is paving the way for a holistic definition of biological age and the development of personalized healthy ageing strategies. This review highlights how the omics technologies and computational modelling are transforming ageing biology into strategies for personalized healthy ageing.

1. Introduction

Biological ageing is a complex, multifactorial process characterised by genetic predisposition, environmental influences and social determinant factors (Hong et al., 2025; Shen et al., 2024a). It involves progressive, non-linear physiological and molecular changes, with people ageing at different rates and through different ageing pathways. While some people experience accelerated biological ageing, in which the functional age of organs and tissues exceeds chronological age, others age more slowly (Chen et al., 2024; Nie et al., 2022; Sun et al., 2021). This variability reflects different ageing patterns or ageotypes, which may be associated with an increased risk of certain chronic diseases (Ahadi et al., 2020). The human lifespan is limited by the ageing process, which is associated with physical decline and an increase in chronic diseases (Chen et al., 2024). However, the causal relationships between age-related mechanisms, health outcomes and the underlying molecular pathways are still incompletely understood. Measuring physiological age independently of chronological age provides a valuable approach to assess individual health risks, including morbidity and mortality (Sun et al., 2021). Identifying person-specific, time-dependent biomarkers of ageing and specific aegotypes associated with increased disease risk could further enhance monitoring and enable targeted interventions to modulate ageing patterns.

The terms ‘biological ageing’, ‘biological age’, ‘ageing biology’, and ‘biological clock’ remain debatable. Following a debate at the Biology of Aging Symposium in 2019, a survey of scientists in the field of ageing revealed a lack of consensus on the definition of ageing, the mechanisms underlying ageing, and whether ageing can be measure or quantified (Cohen et al., 2020). The majority agreed that ageing cannot be measured by a single metric but is multidimensional and heterogeneous. Most also agreed that lifestyle interventions and control of known ageing pathways are likely to slow ageing. Interestingly, there was no consensus on when ageing begins. In a recent paper, Johnson and Shokirev proposed that biological age is an abstract and somewhat qualitative concept (Johnson and Shokhirev, 2024), and that biological clocks should be designated according to the input data, for example, epigenetic data – epigenetic clock. A comparison of different types of biological clocks (telomere length, epigenetic, transcriptomic, proteomic, and metabolomic clocks) revealed poor correlation between the clocks (Jansen et al., 2021), raising the question of which clock should contribute most to the understanding of ageing.

An overview of contemporary theories of ageing identified two major groups: error-based and programme-based theories (De Magalhães, 2025). Error-based theories of ageing are based on the continuous accumulation of biological errors or damage from internal or external insults, driven by random or stochastic processes. These are divided into molecular-driven (DNA damage, telomere attrition, epigenetic changes, *etc.*) or cell-driven (cellular senescence, immune system dysfunction, *etc.*) accumulation of errors. Program-based theories propose that ageing may be programmed, for example, the neuroendocrine theory suggest that hormones regulating reproduction also control ageing.

The study of ageing has attracted considerable attention due to its close relationship with chronic, age-related diseases (Garagnani et al., 2021). This emphasises the urgent need to decipher the molecular mechanisms and pathways that determine the pace and progression of biological ageing. Identifying potential therapeutic targets is a crucial step towards extending life expectancy and facilitating personalised recommendations and interventions to slow down the ageing process (Hong et al., 2025), including dietary changes and physical activity. As the world’s population ages, the associated health and economic burdens are increasing. Maintaining healthy ageing has therefore become a key public health priority (Chen et al., 2025). Interventions targeting biological ageing, especially when implemented from midlife onwards, may represent a promising strategy to reduce the long-term impact of age-related diseases and functional decline.

Recent advances in high-throughput omics technologies and their increasing accessibility have enabled comprehensive studies of age-related molecular changes at the systems level. Their key findings are shown in Fig. 1. While many studies have used omics profiling to explore the biology of ageing, they have focused on linear trajectories of age-related changes, largely overlooking the inter-individual heterogeneity of ageing processes (Shen et al., 2024a). However, thanks to the integrative potential of multi-omics approaches, it is now possible to identify person-specific ageing trajectories and biomarkers, that enable a more refined and personalised understanding of biological ageing.

In this review, we summarise the latest findings from omics studies on biological ageing, with a particular focus on evidence-based interventions involving physical activity and nutrition. In addition, we explore the use of computational tools such as machine learning and artificial intelligence (AI) in deciphering multilevel clinical trials and advancing the development of a new generation of biological clocks. We believe that combining different molecular, physiological, and lifestyle

markers of ageing using explainable AI tools will bring us closer to more accurate predictions of individual biological age and ageing trajectories.

2. Genomics and its role in ageing

People with extreme longevity are a prime example of healthy ageing. Centenarians carry protective variants and a lower burden of risk alleles for common age-related diseases (Garagnani et al., 2021; Tesi et al., 2021; Hao-Tian Wang et al., 2024). The identification of such variants offers insights into the molecular mechanisms of exceptional longevity and strategies to prevent age-related diseases. Longevity is partly hereditary: siblings of centenarians are significantly more likely to live to an advanced age – for example, siblings of individuals who live to 105 are more than 35 times more likely to live to an advanced age than those in the general population (Sebastiani et al., 2016). The heritability of life expectancy up to 70 years is modest (10–25 %), but increases to ~60 % among centenarians, suggesting that exceptional longevity is strongly dependent on favourable genetic factors (Tamvaka et al., 2024; Tesi et al., 2021).

2.1. Telomeres and their role in ageing

Telomeres ensure the stability of DNA and chromosomes, but shorten progressively with cell division, with considerable inter-individual variation (Park et al., 2021; Sun et al., 2021). Telomere attrition is a process that occurs during normal cellular ageing and is part of the biological process known as the cellular senescence (Eppard et al., 2024). Cellular senescence is a component of cellular ageing, and senescent cells are present in many tissues, where they play a role in tissue repair. As cells divide, telomeres shorten, which limits the potential for cellular division and tissue regeneration. Telomere attrition is a regulated process influenced by the presence of inflammation and oxidative stress (Schellnegger et al., 2024).

Women generally have longer telomeres, which may contribute to their longer life expectancy compared to men (Tesi et al., 2021). However, the biological basis of sex differences in life expectancy remains unclear, and the nature of the relationship between ageing and comorbidities is complex. Accelerated telomere attrition is associated with numerous diseases, including Alzheimer's disease, coronary heart disease, and diabetic nephropathy. A genetic predisposition to shorter telomeres increases the risk of chronic kidney disease, while renal dysfunction accelerates telomere loss, reflecting a bidirectional link (Park et al., 2021). Reactivation of telomerase or alternative lengthening of telomeres is a hallmark of multiple cancers, such as glioblastoma, hepatocellular carcinoma, colorectal, breast, prostate, and gastric cancers, as well as haematological malignancies (Sung and Hwang, 2026). Genetic predisposition for longer telomeres may increase the risk of certain blood cancers (Giaccherini et al., 2020). An analysis of telomere length in the UK Biobank prospective cohort revealed an association between telomere length and several haematological malignancies (Li et al., 2024).

Interestingly, telomere length is not conserved between humans and the house mouse (*Mus musculus*), limiting its use in human ageing research. It is evident that telomere length varies not only between mammals but also between individuals, cell types, and even chromosomes. Analysis of telomere length in the blood leukocytes of the house mouse showed that they do not shorten with age, on the contrary, they appear to lengthen (Smoom et al., 2025). Therefore, in mice, telomere shortening does not appear to contribute to the ageing phenotype.

2.2. Genomic insights into longevity and healthy ageing

Genetic variability has traditionally been studied using Genome-Wide Association Studies (GWAS), due to cost efficiency. Early GWAS of centenarians identified *APOE, CDKN2A/B* and *FOXO3* as important longevity loci (Gurinovich et al., 2021). Other loci, such as *CFI/GAR1* and *LINC00202* loci have been associated not only with the rate of physiological ageing but also to age-related traits and diseases (Sun et al., 2021). Recently, a multivariate age-related GWAS of 1.9 million Europeans identified 52 independent variants at 38 loci, 20 of which were novel. These included exonic variants at *LPL* and loci associated with metabolic and brain traits. Genes such as *VEGFA* and *PHB1* were highlighted as important ageing-related candidates (Rosoff et al., 2023).

Extending this approach, Wen et al. (2024) analysed biological age in nine organ systems in > 370,000 UK Biobank participants and identified almost 400 loci. Many were organ-specific, but pleiotropic effects suggested interorgan connections. These findings illustrate how common genetic factors in age-related diseases could lead to organ health

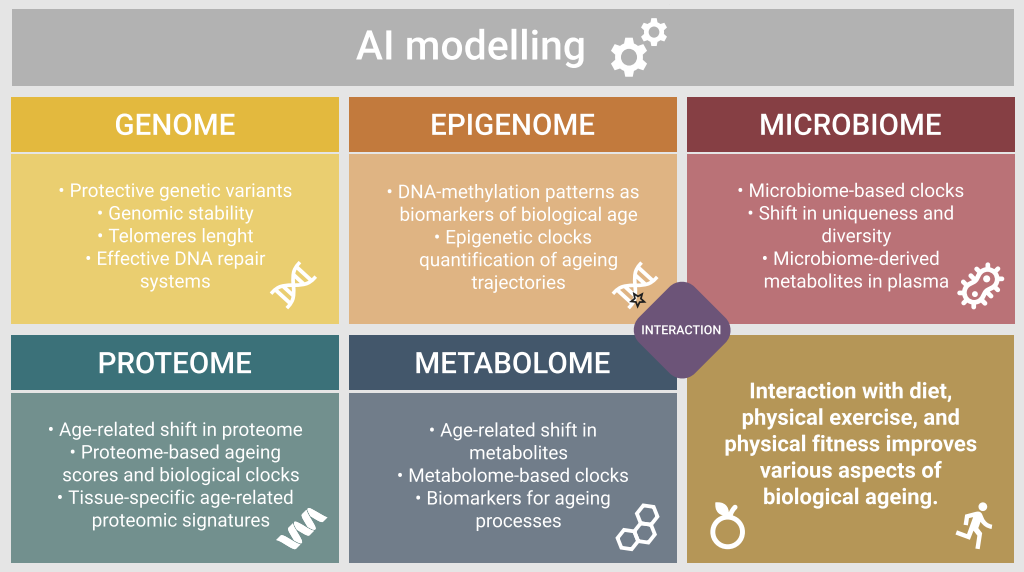

Fig. 1. Overview of biological ageing in the fields of multi-omics and lifestyle. Key findings from genomic studies identify protective genetic variants, genomic stability and DNA repair mechanisms as crucial factors, while epigenomic, proteomic, metabolomic and microbiome profiles serve as biomarkers for ageing. Diet, physical activity and fitness influence these processes and can slow down epigenetic ageing. Computational modelling and AI integrate multi-omics and lifestyle data to predict biological age, assess risk and support personalised prevention strategies. AI, Artificial Intelligence.

interventions, lifestyle strategies, and drug repurposing (Wen et al., 2024).

A large meta-analysis of GWAS identified significant associations of the *APOE* and *GPR78* loci with longevity-related phenotypes across several populations with different ethnic backgrounds and ethnic groups. Interestingly, these loci showed opposite directions of effect on the odds of becoming long-lived (Table 1). Consistent with previous reports, the rs429358 variant of *APOE* (ε4 allele) was associated with lower odds of longevity, whereas the rs7412 variant (ε2 allele) showed the opposite, protective effect, and was reported for the first time in this context. Gene-level association analyses further demonstrated that tissue-specific expression of multiple genes, most of them located near *APOE*, plays an important role in human longevity and is associated with increased odds of exceptional survival. Moreover, longevity shows genetic correlation with several diseases and traits, including coronary artery disease and type 2 diabetes (Deelen et al., 2019a). Although several GWAS have identified multiple candidate variants associated with lifespan, replication across independent cohort studies remains limited. Among all reported loci, variants in *APOE*, *FOXO3* and *CDKN2A/B* remain the most consistently replicated genetic determinants of human longevity and lifespan (Broer et al., 2015; Deelen et al., 2019a; Gurinovich et al., 2021).

Despite progress, most studies still focus on common variants.

Table 1
An overview of candidate variants linked to longevity and lifespan.

Gene name/ Closest gene	Variant	Association with longevity/lifespan/age-related diseases	Reference
APOE	rs429358 (ε4 allele)	Lower odds of longevity, increased risk of various age-related diseases.	(Deelen et al., 2019a; Timmers et al., 2019)
	rs7412 (ε2 allele)	Higher odds of longevity, decreased risk for several age-related diseases.	(Deelen et al., 2019a)
FOXO3	rs2802292	Higher odds of longevity.	(Deelen et al., 2019a; Morris et al., 2015)
	rs12206094	Higher odds of longevity.	(Flachsbart et al., 2017)
	rs4946935	Higher odds of longevity.	(Flachsbart et al., 2017)
CDKN2A/B	rs2184061	Increased parental lifespan.	(Deelen et al., 2019a; Gurinovich et al., 2021)
	rs1556515	Parental longevity.	(Gurinovich et al., 2021)
	rs1556516	Lower odds of longevity and coronary artery disease.	(Deelen et al., 2019a; Timmers et al., 2019)
GPR78	rs7676745	Lower odds of longevity.	(Deelen et al., 2019a)
IL-6	rs2069837	Lower odds of longevity.	(Deelen et al., 2019a; Zeng et al., 2016)
ANKRD20A9P	rs2440012	Remains to be characterized.	(Deelen et al., 2019a; Zeng et al., 2016)
CFI/GAR1	rs11940869	Physiological ageing rate, ageing, age-related diseases.	(Sun et al., 2021)
LINC00202	Not stated	Physiological ageing rate, ageing, age-related diseases.	(Sun et al., 2021)
LPL	rs268	May affect healthy ageing.	(Rosoff et al., 2023)
STK17A/COA1	rs7456688	Higher odds of longevity.	(Garagnani et al., 2021)
LHFPL6	rs9576827	Compensatory mechanisms counterbalancing age-related decline.	(Gurinovich et al., 2021)

Increasingly, rare variants are coming into focus, especially in cases of extreme longevity. For example, the CALERIE Genomic Data Resource provides whole-genome genotypes, DNA methylation, mRNA, and small RNA data from blood, adipose tissue, and skeletal muscle to explore how caloric restriction affects biomarkers of ageing and is discussed in more detail in Chapter *7. The Role of Nutrition in Regulating Biological Age* (Ryan et al., 2024).

Whole genome sequencing of semi-supercentenarians and supercentenarians (105 + and 110 + years) has revealed unique genetic profiles. In addition to improved DNA repair, clonal haematopoiesis was shown to be a protective factor against cardiovascular events. The *STK17A* gene showed the strongest association with longevity, while the rs7456688-A allele between *STK17A* and *COA1* characterised many 105-year-old survivors (Garagnani et al., 2021). Similarly, long-lived individuals exhibit efficient DNA repair and genomic integrity, which is associated with protection against somatic mutational load (Hao-Tian Wang et al., 2024).

Somatic mutation analyses further underline the genomic stability with extreme longevity. In Chinese centenarians, mutations clustered non-randomly, preserving key genomic regions (Hao-Tian Wang et al., 2024). In men, mosaic loss of chromosome Y is the most common somatic alteration and has been linked to genome instability, reduced life expectancy and increased risk of cancer, cardiovascular disease (CVD), and Alzheimer's disease (Guan et al., 2024).

Longevity variants also correlate with proteomic signatures, as they have been identified as protein quantitative trait loci (pQTLs) that correlate with distinct proteomic signatures, emphasising their potential for identifying specific targets of healthy ageing. They may promote healthy ageing by reducing the accumulation of damage or enhancing compensatory mechanisms. For example, carriers of *APOE* ε2 have lower APOB levels, while *CDKN2B* longevity alleles are associated with lower GDF15, both favourable biomarkers. Conversely, higher TGFB1 and NENF levels in carriers of certain rare alleles may represent compensatory mechanisms that counterbalance age-related decline (Gurinovich et al., 2021). An overview of gene variants linked to longevity and lifespan is shown in Table 1.

Although genetic determinants, including specific genes and signalling pathways, have been identified, other factors that contribute to longevity and healthy ageing, such as environmental influences (discussed in Chapters *6. Effects of Exercise on Biological Ageing* and *7. The Role of Nutrition in Regulating Biological Age*), are often overlooked. The genetic association with human lifespan is well established, but estimates of longevity heritability remain modest (approximately 15–30 %), indicating that non-genetic factors play a substantial role despite the common belief that genetic factors largely explain familial longevity (Broer et al., 2015; Deelen et al., 2019a; Ruby et al., 2018). For example, research by Graham Ruby *et al.* showed that the heritability of human longevity is below 10 % and has been overestimated due to assortative mating. Significant phenotypic correlations were observed not only among blood relatives but also between spouses and other in-laws, highlighting the importance of assortative mating as a factor affecting longevity. Transferable variance, which includes both genetic contributions and inherited sociocultural factors, was consistently low (below 10 %) when comparing different types of relatives (Ruby et al., 2018). In addition, while some genetic determinants of longevity are common across populations, population-specific variants may have different and important effects on lifespan. By using an integrative approach, this work provides an important systematic and comprehensive insight into different areas of biological ageing.

2.3. Genetics of age-related diseases

Genetic determinants of longevity often overlap with those of age-related diseases. Variants in *APOE* and *ANRIL* influence cardiovascular risk, *CDKN2B* and *BEND4* are associated with cancer, while *FOXO3* and *LPA* are related to diabetes and the stress response (Sun et al., 2021;

Tamvaka et al., 2024; Tesi et al., 2021; Tseng et al., 2023).

Individuals aged ≥ 105 years are often protected from cardiovascular disease, partly due to reduced accumulation of somatic mutations and favourable lipid profiles such as lower APOB levels, consistent with protective *APOE* alleles (Gurinovich et al., 2021). In addition, rare variants in *NME1* (a metastasis suppressor) and *PLEKHG4* (linked to neurodegeneration) have been enriched in centenarians, suggesting a role in delaying cancer and neurological disease (Garagnani et al., 2021).

Cognitive abilities such as processing speed, memory and executive functions are crucial for maintaining independence in old age. As these abilities decline with age, cognitive resilience is increasingly recognised as critical to healthy ageing. GWAS have linked loci involved in synaptic function and neurogenesis to the maintenance of cognitive abilities, largely independent of intelligence (Fitzgerald et al., 2022). Feng et al. (2025) highlighted the importance of preserving white matter integrity and brain volume for cognitive performance, implying that protecting certain brain regions could enhance resilience (Feng et al., 2025). A polygenic risk score (PRS) developed by Tesi et al. (2021), comprising 330 variants, successfully distinguished cognitively healthy centenarians from older adults and appeared to mitigate the deleterious effects of APOE ε4. These findings emphasise that genetic resilience involves stress response, neural development, and compensatory pathways (Tesi et al., 2021).

Genetics also influences frailty and mental health. Frailty has been shown to be highly polygenic and genetically linked to cognitive ability, with older people being more susceptible to its development (Tseng et al., 2023). Accelerated biological ageing predicts a higher risk of depression and anxiety, even after controlling for genetic risk, suggesting that the ageing processes themselves may predispose to mental illness in later life (Gao et al., 2023).

Finally, the mitochondrial genome contributes to ageing by accumulating somatic mutations that impair oxidative balance, apoptosis regulation, and cellular homeostasis. mtDNA haplogroups influence the risk of neurodegenerative diseases, including Parkinson's disease and dementia, with both protective and deleterious roles reported (Tamvaka et al., 2024).

To summarise, genetic variability is a key factor in longevity and healthy ageing. Protective variants improve genomic integrity, favour metabolic and immunological balance, and support resistance to cognitive and systemic decline. Both common and rare variants contribute, with population-specific differences shaping outcomes. While remarkable progress has been made, the field still faces challenges with reproducibility and incomplete mechanistic understanding. Future large-scale, multi-ethnic studies integrating genomic, epigenomic, and multi-omic data are essential to uncover robust biomarkers and actionable therapeutic targets.

Boxmary Box 1: Genomics

Centenarians carry protective genetic variants and fewer risk alleles for age-related diseases, which contributes to their exceptional longevity. The latter is highly heritable, with genetics accounting for up to 60 % of lifespan in centenarians, compared to 10–25 % in the general population.

Telomeres and ageing: Telomeres shorten with age and vary by sex, with women generally having longer telomeres, possibly contributing to sex differences in ageing and DNA repair decline. Accelerated telomere shortening is associated with numerous diseases, with a bidirectional relationship between telomere loss and chronic disease.

Genetics and age-related diseases: GWAS have identified key genetic variants associated with longevity and age-related diseases (*e.g. APOE, CDKN2A/CDKN2B, FOXO3, VEGFA, PHB1*), while NGS studies in centenarians reveal common and rare genetic variants that point to DNA repair efficiency as a critical element of healthy ageing. Many variants associated with longevity are also protein quantitative trait loci (pQTLs), which are associated with unique proteomic signatures that may reduce the accumulation of damage or enhance compensatory responses in ageing. In addition, organ-specific and pleiotropic genetic effects emphasise the interconnectedness of biological ageing across multiple systems.

Age-related diseases: Favourable genetic variants reduce the risk of age-related diseases and contribute to a longer lifespan and delayed onset of diseases. Cognitive resilience and brain ageing are influenced by genetic loci related to synaptic function and neurogenesis, with polygenic risk scores helping to identify individuals with preserved cognition. In addition, accelerated biological ageing is associated with a higher risk of depression and anxiety, independent of genetic risk. The age-related accumulation of DNA mutations contributes to neurodegeneration and emphasises the role of genome integrity in healthy ageing.

3. Epigenomics and ageing: the role of DNA methylation

Whole blood is the tissue most frequently analysed for methylation patterns, but other sources such as saliva, semen, bone and teeth have also been investigated. In forensics, specific CpG sites allow accurate differentiation between biological fluids, which can also be applied in medicine and ageing research, where tissue-specific methylation patterns can provide information on biological age (Gerra et al., 2024; Kader et al., 2020; Marcante et al., 2025).

A number of methods have been used to assess DNA methylation, including Sanger sequencing, methylation-specific PCR, methylation-sensitive high-resolution melting, MassARRAY (MALDI-TOF), single-base extension with SNaPshot chemistry, pyrosequencing and next-generation sequencing (NGS) (Marcante et al., 2025). Until recently, almost all studies relied on bisulfite conversion to distinguish between methylated and unmethylated cytosines, mainly by whole genome bisulfite sequencing (WGBS) (Freire-Aradas et al., 2020). Newer technologies now allow direct detection of DNA methylation on a genome-wide scale without the need for bisulfite conversion. These include long-read sequencing platforms such as nanopore sequencing from Oxford Nanopore Technologies (ONT) and single-molecule real-time sequencing (SMRT) from Pacific Biosciences (PacBio) (Sigurpalsdottir et al., 2024).

For example, direct nanopore-based methylation sequencing in individuals with type 1 diabetes and persistent hyperglycaemia identified differentially methylated CpG sites in genes associated with chronic complications, highlighting their potential for the study of age-related epigenetic changes (Čugalj Kern et al., 2024). Furthermore, simultaneous profiling of short tandem repeats (STRs), single nucleotide polymorphisms (SNPs), insertions/deletions (InDels), mitochondrial DNA (mtDNA) and DNA methylation markers enables comprehensive analysis for methylation-based age prediction. The detection of genetic variants showed 90–100 % agreement with validation data, while methylation markers showed strong correlations with both validation data and chronological age (De Bruin et al., 2025).

3.1. Development and utility of epigenetic clocks

A growing number of studies investigating biological ageing now rely on DNA methylation analysis as the central method for age estimation, often in combination with other techniques. Epigenome-wide association studies (EWAS) have become particularly important as they enable the identification of methylation-based biomarkers and provide mechanistic insights into the ageing process (Christofidou and Bell, 2025; Marcante et al., 2025). As CpG methylation (5-mCpG) is the predominant DNA modification in humans and plays a key role in regulating gene expression, it has become a major focus of research as a biomarker of ageing (Gerra et al., 2024). Numerous studies have shown that the degree of methylation at specific CpG sites is strongly correlated with chronological age. This observation led to the development of first-generation epigenetic clocks, including that of Hannum and Horvath in 2013, which estimate biological age based on DNA methylation

sites. The Horvath clock, based on 353 CpG sites, is a multi-tissue predictor, while the Hannum clock uses 71 CpG sites and performs best in whole blood (Bell et al., 2019; Hannum et al., 2013; Horvath, 2013). The CpG sites for both predictors were selected using a similar penalised regression model, yet they only have six CpG sites in common (Jylhävä et al., 2017). Finding that gaps between predicted and actual age reflect biological ageing led to second-generation clocks such as PhenoAge, which links clinical biomarkers to 513 CpG sites, and GrimAge, which integrates plasma proteins and smoking history to identify 1030 CpGs that predict mortality (Levine, 2020; McCrory et al., 2021). More recently, third-generation clocks such as DunedinPoAm and DunedinPACE have been developed using longitudinal cohort data to quantify the rate of biological ageing independent of chronological age (Belsky et al., 2022, 2020; Christofidou and Bell, 2025; Marcante et al., 2025; Nwanaji-Enwerem et al., 2025). The call for robust multi-tissue predictors aiming to capture systemic rather than tissue-restricted ageing has driven validation of existing clocks in other tissues. PhenoAge (Levine et al., 2018) was developed in blood but validated across multiple tissues including brain, liver, kidney, lung and fibroblasts; GrimAge (Lu et al., 2019) remains largely blood-based with limited cross-tissue use; and DunedinPoAm/DunedinPACE (Belsky et al., 2022) were derived from blood cohorts but are now being expanded to other tissues. Using DNA methylation data from umbilical cord blood and neonatal blood samples, an epigenetic clock was developed to estimate gestational age at birth, identifying 148 CpG sites predictive of gestational age and showing high concordance with clinical estimates such as ultrasound measurements (Knight et al., 2016). In addition to conventional telomere length assessments, DNAmTL provides an estimate of telomere length based on DNA methylation patterns (Eisenberg et al., 2024). Technical variability in methylation assays can reduce the accuracy of epigenetic clocks, with differences of up to 9 years observed across technical replications. Retraining on principal components (PC) reduces this error to about 1 year, improving the reproducibility of widely used clocks such as Horvath1, Horvath2, Hannum and PhenoAge (Higgins-Chen et al., 2022). Similar improvements have been confirmed across platforms, underscoring the robustness of PC-trained clocks for research and clinical applications (Shokhirev and Johnson, 2025). Summary of key epigenetic clocks, their applications, and limitations are shown in Table 2.

3.2. DNA methylation as a human lifespan biomarker

Building on this, recent studies have integrated DNA methylation detection with epigenome-wide association study approaches and epigenetic ageing biomarkers to investigate developmental and ageing trajectories, as well as health outcomes, from birth to old age. One such example is preterm birth, which has been linked to increased risk of chronic diseases later in life, where epigenetic memory may contribute to long-term disease susceptibility. In this context, Kashima et al. (2021) examined gestational age (GA)-related epigenetic memory by analysing DNA methylation using both sequencing and microarray platforms in preterm and term infants. The study identified CpG sites and transcripts associated with GA, including 54 CpG sites showing coordinated methylation and transcriptional changes. These findings support the hypothesis that GA-associated epigenetic memory contributes to health outcomes in individuals born preterm (Kashima et al., 2021). In children from birth to age 10, Mallisetty et al. (2020) conducted an epigenome-wide association study to evaluate the influence of infant exclusive breastfeeding, exclusive formula feeding and mixed feeding on DNA methylation. Using the Illumina 450 K array, they identified 87 CpG sites significantly associated with feeding mode. Notably, exclusive formula feeding in children exhibited greater overall declines in DNA methylation and exert long-term epigenetic effects relevant to child development and health (Mallisetty et al., 2020). Chen et al. (2020) performed a post hoc analysis of a randomized controlled trial to evaluate the impact of maternal vitamin D_3 supplementation on epigenetic gestational age acceleration (GAA) in new-borns. DNA methylation from cord blood in 92 pregnant women was analysed using the Illumina EPIC array, and GAA was estimated using the Knight and Bohlin clocks. Although no significant effects were observed in the overall cohort, vitamin D_3 supplementation significantly reduced GAA in African American infants, suggesting a potential protective effect against accelerated epigenetic ageing at birth (Chen et al., 2020). In the context of noncommunicable diseases, Shiau et al. (2021) assessed the effect of prenatal exposure to gestational diabetes mellitus (GDM) on offspring epigenetic ageing in a cohort of 578 GDM-exposed and 578 non-exposed mother–child pairs. DNA methylation was measured at a median child age of 5.9 years using the Illumina 850 K array. GDM exposure was associated with significantly accelerated epigenetic age, as estimated by Horvath and Hannum clocks implicating epigenetic mechanisms in the developmental origins of health and disease (Shiau et al., 2021). In adults, Yaskolka Meir et al. (2023) examined the effects of polyphenol-rich diets on biological ageing involving 256 adults with abdominal obesity or dyslipidaemia. DNA methylation-based biological age was assessed using multiple epigenetic clocks, including DunedinPACE, Horvath, Hannum, and Li clocks, via Illumina EPIC arrays. No significant differences were seen between diet groups overall, higher adherence to the polyphenol-rich diet was linked to significantly slower epigenetic ageing, suggesting diet as a modifiable factor for age-related health outcomes (Yaskolka Meir et al., 2023). Vyas et al. (2023) explored associations between epigenetic ageing markers and cognitive outcomes in 45 older adults over a 2-year period using the Illumina EPIC

Table 2
Overview of major epigenetic clocks, highlighting their practical applications, limitations, sensitivity to biological and environmental factors.

Clock	Generation	Usefulness / Strengths	Limitations	Sensitivity	Reference
Hannum	1st-generation; 71 CpGs	Accurate in whole blood; simple design; strongly correlated with chronological age.	Blood-specific; poor generalization to other tissues.	Sensitive to blood cell composition and lifestyle factors.	(Hannum et al., 2013)
Horvath	1st-generation; 353 CpGs	Multi-tissue predictor; widely used reference; strong correlation with age.	Slightly less precise in blood vs Hannum; some tissue variability.	Captures cross-tissue effects; sensitive to environmental exposures.	(Horvath, 2013)
PhenoAge	2nd-generation; 513 CpGs	Predicts morbidity and mortality; links to healthspan.	Primarily trained in blood; limited validation in non-blood tissues.	Sensitive to clinical biomarkers and environmental factors.	(Levine et al., 2018)
GrimAge	2nd-generation; 1030 CpGs	Strong predictor of mortality and disease; integrates DNAm surrogates for plasma proteins + smoking.	Blood-based; complex design; limited cross-tissue validation.	Captures smoking history, protein biomarkers.	(Lu et al., 2019)
DunedinPoAm / DunedinPACE	3rd-generation; longitudinal pace measure	Quantifies rate of ageing; sensitive to interventions and exposures.	Validated mostly in blood; expansion to other tissues ongoing.	Sensitive to lifestyle, environment, and disease.	(Belsky et al., 2022)
DNAmTL	Derived telomere estimator	Proxy for telomere length; correlates with cellular ageing.	Lower predictive value for lifespan vs PhenoAge/GrimAge; tissue-specific.	Sensitive to replicative ageing.	(Eisenberg et al., 2024)

arrays, with biological age estimated via GrimAge, PhenoAge, and DNAm-based telomere length (DNAmTL). Higher GrimAge was associated with cognitive decline, whereas longer DNAmTL predicted better cognitive performance underscoring the potential of epigenetic biomarkers for cognitive and neuropsychiatric ageing trajectories (Vyas et al., 2023).

Despite advances, translation of DNA methylation and other omics-based biomarkers into clinical practice remains limited. High-throughput assays such as Illumina EPIC arrays (Chen et al., 2020; Mallisetty et al., 2020; Yaskolka Meir et al., 2023) and emerging long-read sequencing platforms including ONT and PacBio (Sigurpalsdottir et al., 2024) remain costly and require specialized infrastructure, restricting scalability beyond research settings. Cross-platform variability, preprocessing pipelines, and batch effects introduce technical heterogeneity that complicates meta-analysis and cross-study comparability (De Bruin et al., 2025; Freire-Aradas et al., 2020). Although PC-trained clocks have substantially improved reproducibility (Higgins-Chen et al., 2022; Shokhirev and Johnson, 2025), the absence of standardized protocols, validated reference datasets, and consensus thresholds for biological age interpretation continues to impede clinical adoption (Gerra et al., 2024; Marcante et al., 2025). Addressing these barriers in cost, accessibility, and methodological standardization will be critical for transition from research to healthcare applications.

All things considered, methylation patterns make it possible to distinguish between different tissues and biological fluids and provide information about their biological age. Over time, several generations of epigenetic clocks have been developed to predict biological age, including clinical and biochemical parameters, including social determinants, in addition to CpG sites. In addition, studies emphasise the value of DNA methylation as a biomarker across the human lifespan by linking early life exposures, dietary factors and chronic disease risk to epigenetic ageing trajectories. The observed heterogeneity in methylation change across individuals further suggests that distinct ageing pathways may exist, a perspective discussed in recent work on metabolic and proteomic ageing patterns, which may also extend to epigenetic domains (Ahadi et al., 2020).

Boxmary Box 2: Epigenetics

DNA methylation, the most extensively characterized and arguably the most critical epigenetic modification, is a primary regulator of transcriptional activity and has emerged as a highly reproducible biomarker of biological ageing.

Methods and advances: Techniques for methylation analysis range from PCR and pyrosequencing to large-scale bisulfite sequencing and arrays. New long-read sequencing platforms (ONT, PacBio) allow direct methylation detection without chemical conversion, improving accuracy and enabling forensic and medical applications.

Environmental and health significance: Epigenetic ageing is influenced by both early-life conditions and adult social determinants. Factors related to birth, nutrition, and behaviour can shape DNA methylation patterns, while choices in diet, activity, and health habits later in life affect the pace of ageing. As a result, DNA methylation is emerging as a versatile biomarker that links ageing with disease risk, cognitive outcomes, and overall health, with growing potential in research, clinical practice, and forensic applications.

4. Proteomic signatures of ageing plasma

Proteomic analysis of blood samples is now commonly used to discover various biomarkers of physiological and pathophysiological processes. Sampling of blood is minimally invasive and can be done repeatedly, enabling the study of longitudinal changes in blood proteins. It has already been demonstrated that analysis of plasma protein expression, together with other health-related phenotypic data, can serve as an indicator of health and various health issues (Williams et al., 2019).

4.1. Age-related changes in blood proteome

Several studies have confirmed that blood proteins are significantly associated with various age-related parameters. Shortening of telomere length is a hallmark of biological ageing. A proteome-wide Mendelian randomisation analysis was conducted on two cohorts: the deCODE Health Study and UK Biobank, aiming to identify protein biomarkers associated with telomere length (Zhao et al., 2024). The analysis identified 22 plasma proteins causally associated with telomere length; however, five (APOE, SPRED2, MAX, RALY, and PSMB1) showed the highest evidence of association.

A study combining four datasets (ABF300, HERITAGE, LonGenity, and deCODE) of plasma proteomics identified 273 plasma proteins (termed ageing proteins) significantly associated with ageing (Coenen et al., 2023). Plasma levels of 196 proteins increased, while 77 decreased. Additionally, 139 of these proteins were found to be associated with age in at least one other published study. Functional enrichment analysis confirmed that multiple processes are affected by ageing, such as glycosaminoglycan binding and extracellular matrix organisation. Clustering analysis of all detected proteins according to their expression trajectories identified 15 clusters with a wide diversity of enriched processes, including extracellular matrix, regulation of neurogenesis, and complement and coagulation cascades. The top 20 ageing proteins were associated with several phenotypes, especially those related to ageing, such as frailty, multimorbidity and mortality. Among them were novel proteins: WAP four-disulphide core domain protein 2 (WFDC2), Trafficking protein particle complex subunit 3 (TRAPPC3), Macrophage scavenger receptor types I and II (MSR1) and CUB domain-containing protein 1 (CDCP1). Finally, they identified ageing proteins that may be associated with either accelerating or decelerating the ageing process, or may play a modulatory role in ageing (Coenen et al., 2023).

Plasma proteomic analysis of Finnish participants comparing two cohorts with median ages approximately 22 and 62 years, identified eight proteins associated with three or more epigenetic age acceleration (EAA) estimates (Drouard et al., 2025). These proteins are S100-A8, S100-A9, C-reactive protein (CRP), complement component C9 (C9), leucine-rich alpha-2-glycoprotein (LRG1), myeloperoxidase (MPO), and receptor-type tyrosine-protein phosphatase S (PTPRS). MMP9 (matrix metallopeptidase 9) was the only protein associated with six EAA estimates. EAA was defined as the residuals of chronological age regressed on epigenetic age, and was calculated using six different algorithms to estimate biological ageing from DNA methylation: Horvath (Horvath, 2013), Hannum (Hannum et al., 2013), GrimAge (Lu et al., 2019), GrimAge2 (Lu et al., 2022), PhenoAge (Levine et al., 2018) and DunedinPACE (Belsky et al., 2022). Interestingly, a pairwise association of co-twins in the FinnTwin12 cohort found that the closer the plasma proteomes of the two co-twins were, the more similar the epigenetic age accelerations between the twins were detected (Drouard et al., 2024).

A two-sample Mendelian randomisation analysis was used to identify proteomic signatures of plasma proteomic biomarkers from the UK Biobank Pharma Proteomics Project cohort and multidimensional ageing phenotypes (Cao et al., 2025). These phenotypes include Klemera and Doubal's method biological age (KDM-BA) acceleration, PhenoAge acceleration, frailty index, leukocyte telomere length (LTL), and health span. In total, 71 protein biomarkers were found genetically linked to multidimensional ageing phenotypes and 12 of these were validated in the FinnGen cohort. Next, 12 proteins achieved nominal significance with parental lifespan. These were APOE, FURIN, SDCCAG8, CELSR2, HLA-E, ZPR1, CEP170, CD46, ASGR1, UMOD, CXCL8, and ATXN2L. Enrichment analysis confirmed that the 71 genes are involved in immune response and the regulation of cell adhesion pathways. Phenotypic association between the plasma proteome and ageing revealed 1801 proteins significantly associated with chronological age. Phenotype-wide association analyses showed that 64 out of the 71 identified proteins had at least one significant association with a

PheCODE diagnosis. The study identified a set of proteins associated with ageing, including UMOD, HEXIM1, ACP1, EFEMP1, ATXN2L, FURIN, CELSR2, APOE, NT5C3A, IL1RN, KIT, SERPINF2, CEP170, SERPINA1, ARFIP1, NFATC1, TRDMT1, PARP1, TK1, TYMP, RPA2, and COMMD1, which are known for their roles in immune response and inflammation processes.

Studies in centenarians have also confirmed that blood protein expression is a valuable source of ageing-related biomarkers. Protein serum signatures can distinguish between centenarians, their offspring, and age-matched controls of the offspring, as discovered in the New England Centenarian Study (NECS) (Sebastiani et al., 2021). In this study, researchers found 1312 proteins with expression levels that differed between centenarians and the younger group. Functional analysis of these proteins revealed pathways involved in immune system and cell cycle. Interestingly, they discovered that centenarians acquire similar ageing signatures, but do so at a later age, which supports the hypothesis that centenarians age at a slower rate. A study in long-lived Sicilians also found correlations between protein expression in plasma and age, identifying proteins that increase or decrease with age (Siino et al., 2022).

Longitudinal studies of the plasma proteome have identified age-related patterns in protein expression. By analysing the plasma proteome of UK Biobank participants, 227 proteins involved in inflammation and regeneration pathways were found to be significantly associated with ageing (Ma et al., 2025). The analysis revealed age-related shifts in the proteome, peaking at 41, 60 and 67 years of age. Mendelian randomisation identified causal links between ageing and five proteins (CXCL13, DPY30, FURIN, IGFBP4, SHISA5), which were highlighted as promising biomarkers for ageing. A multiomics longitudinal study revealed nonlinear patterns in molecular markers of ageing, with major dysregulation occurring at approximately 44 and 60 years of age (Shen et al., 2024b). Plasma proteomic analysis showed that 26 proteins changed significantly during ageing and exhibited two age-related waves at around 40 and 60 years. Comparison of changes in the transcriptome, metabolome, and gut, skin, and nasal microbiome further supported the hypothesis that ageing-related changes are not limited to a specific omics layer. Biological ageing involves coordinated and systemic changes across multiple molecular components.

4.2. Use of proteome in age-related clocks and scores

Plasma protein biomarkers can be used to calculate various ageing proteome scores or age-related clocks. A systematic review and analysis of 23 proteomic studies published up to 2020 identified 1128 proteins that change with age and are involved in inflammation, extracellular matrix, and gene regulation (Johnson et al., 2020). The authors proposed a 23-plasma protein panel to serve as a proteomic ageing clock. Expression levels of plasma proteins and DNA-methylation signatures from four different cohorts were used to train and test EpiScores – epigenetic scores that show a relationship between epigenetic markers and protein abundance (Gadd et al., 2022). One hundred and thirty EpiScores were found to be associated with different morbidities, indicating that epigenetic scores based on plasma protein levels could predict an individual risk of various diseases as they age. An important aspect of healthy ageing is also the delay of common age-related diseases. Proteomic data from the UK Biobank cohort were used to develop ProteinScores to predict the risk of age-related diseases (Gadd et al., 2024). This study highlighted that appropriate risk stratification could identify individuals before formal diagnoses. It also emphasised that scoring systems should be developed on a disease-by-disease basis.

A comparison of different biological clocks revealed poor correlation and highlighted the need to measure biological ageing at multiple cellular levels (Jansen et al., 2021). The study, conducted on the Netherlands Study of Depression and Anxiety (NESDA) cohort, found significant correlations between proteomic and metabolomic ageing, as well as transcriptomic and proteomic ageing. Alcohol use, smoking, and high body mass index (BMI) were associated with advanced proteomic ageing in this study.

Comparing fifteen omics ageing clocks, including the PEA proteomics clock, from several cohorts concluded that it is possible to construct accurate estimations of ageing clocks by using a variety of omics biomarkers (Macdonald-Dunlop et al., 2022). Their data analysis also indicated that there may be more than one type of biological age, and that few omics could be effective in predicting risk factors.

4.3. Evaluation of organ-specific ageing using proteome

Proteome analysis supports the organ-specific ageing approach as one method to assess individual ageing. Plasma proteomics analysis was used to study organ-specific ageing in the HELPFul cohort, searching for sex-related associations and links with cardiovascular diseases (Qu et al., 2025). The study demonstrated that plasma proteomics can determine proteome-derived ageing and identified sex-related differences in organ ageing. It confirmed that diabetes and a higher triglyceride-glucose BMI are associated with faster organ ageing.

A longitudinal study of plasma proteomics, metabolomics, transcriptomics, and gut and nasal microbiomes proposed four ‘ageotypes’ based on changes in molecular pathways in four different compartments: immunity, metabolism, liver dysfunction, and kidney dysfunction (Ahadi et al., 2020). All ageotypes were defined by significant time-related changes in cytokines and other proteins, as well as in metabolites, transcripts, and clinical laboratory values.

Blood proteomics is a valuable tool for determining ageing biomarkers in individual tissues. The serum proteome has been shown to significantly associate with bone mineral – an important factor in the development of the age-related disease osteoporosis – and protein expression levels were used to generate the biological age of bone in the Guangzhou Nutrition and Health Study (GNHS) (J. Xu et al., 2024). A study of ageing skeletal muscle revealed a distinct proteomic profile between young and old men (Gueugneau et al., 2021). The proteomic analysis showed a fast-to-slow transition, and downregulation of glycolysis in older skeletal muscles. A study of brain-ageing biomarkers in the UK Biobank identified 13 plasma proteins significantly associated with brain ageing (Liu et al., 2024). The researchers also identified three protein expression waves associated with brain age at 57, 70, and 78 years, suggesting these are critical periods for intervention in brain ageing processes.

Differences in blood proteins are a valuable source for calculating biological age, as in many studies. Blood proteins are significantly associated with various age-related parameters. Their expression levels can be used to calculate different age-related scores and clocks; however, it appears that scoring systems should be developed on a disease-by-disease basis. Longitudinal studies confirm age-related changes in protein levels with age-related peaks in protein expression. Studies in centenarians have confirmed the hypothesis that they age at a slower rate. Combining proteomic data with different omic layers has revealed different types of biological ageing. The conclusion is that biological ageing involves coordinated and systemic changes across multiple molecular components, including the proteome.

A comprehensive and dynamic atlas of protein expression across human tissues over five decades revealed widespread decoupling of the transcriptome and proteome, as well as a decline in proteostasis (Ding et al., 2025). Organ-specific protein signatures reflecting different temporal changes in ageing were identified. Downregulation of proteins involved in mitochondrial biogenesis, homeostasis and respiration was recognised as a hallmark of ageing. YTHDC1 (YTH N6-Methyladenosine RNA Binding Protein C1) emerged as a consistently downregulated epigenetic regulator across tissues. Comparison of proteomic age clocks in thirteen different tissues revealed asynchronous ageing. Alterations in endocrine homeostasis were found to be among the initiating events of systemic ageing, while the aorta showed early ageing and marked sensitivity to ageing. Finally, circulating age-accumulating senoproteins

were identified, cytokines released by senescent tissues or cells were collectively labelled as senokines (CXCL12, GAS6, *etc.*), driving vascular and systemic ageing.

Boxmary Box 3: Proteomics

Proteomics is an important method for identifying blood biomarkers of physiological and pathological processes, offering a minimally invasive and repeatable way to monitor age-related molecular changes in blood proteins. For example, plasma protein signature can indicate overall health status and risk for age-associated diseases.

Age-related changes in blood proteome: Numerous blood proteins have been identified as significantly associated with age-related parameters, including telomere length, epigenetic age acceleration, chronological age, and clinical phenotypes such as frailty, multimorbidity, and mortality. Studies reveal non-linear trajectories in the plasma proteome, with major shifts occurring around ages 40 and 60. Centenarians exhibit proteomic ageing signatures similar to those observed in the general population, however, these changes emerge later in life, supporting the concept of a slower pace of biological ageing in long-lived individuals.

Use of proteome in age-related clocks and scores: Plasma protein biomarkers can be used to calculate various proteome-based ageing scores or age-related biological clocks, such as EpiScores and ProteinScores, to predict the risk of age-related diseases.

Evaluation of organ-specific ageing using proteome: Blood proteomics is a powerful tool for identifying ageing biomarkers in individual tissues and for uncovering sex-specific differences in organ ageing. Studies have revealed distinct proteomic signatures associated with ageing processes in bone, skeletal muscle, and the brain, providing insights into tissue-specific trajectories and highlighting critical periods of molecular dysregulation across organs.

5. Metabolomic signatures of ageing in blood plasma

Metabolomics is the large-scale study of small molecules, commonly referred to as metabolites, in cells, biological fluids, tissues or organisms. The totality of these small molecules and their interactions within a biological system is referred to as the metabolome. Metabolomics studies can provide biological information on a variety of ageing processes (Panyard et al., 2022). Major metabolic pathways are that are altered with ageing ate presented in Fig. 2. For identifying and quantifying metabolites, advanced analytical techniques, primarily mass spectrometry and nuclear magnetic resonance (NMR) are used (Hajnajafi and Iqbal, 2025; Madrid-Gambin et al., 2023).

5.1. *Age-related changes in the plasma metabolome*

Numerous studies have revealed associations between specific metabolites, particularly those related to lipid metabolism and redox balance, and the ageing process, suggesting their potential utility as biomarkers for biological ageing and longevity (Adav and Wang, 2021; Castro et al., 2022). In particular, the blood plasma metabolome, the complete set of small molecules, or metabolites, present in blood plasma, offers a dynamic reflection of the body's metabolic state. This metabolome is influenced by genetics, diet, medications, and environmental exposures, making it a valuable source of information for understanding physiological changes over time.

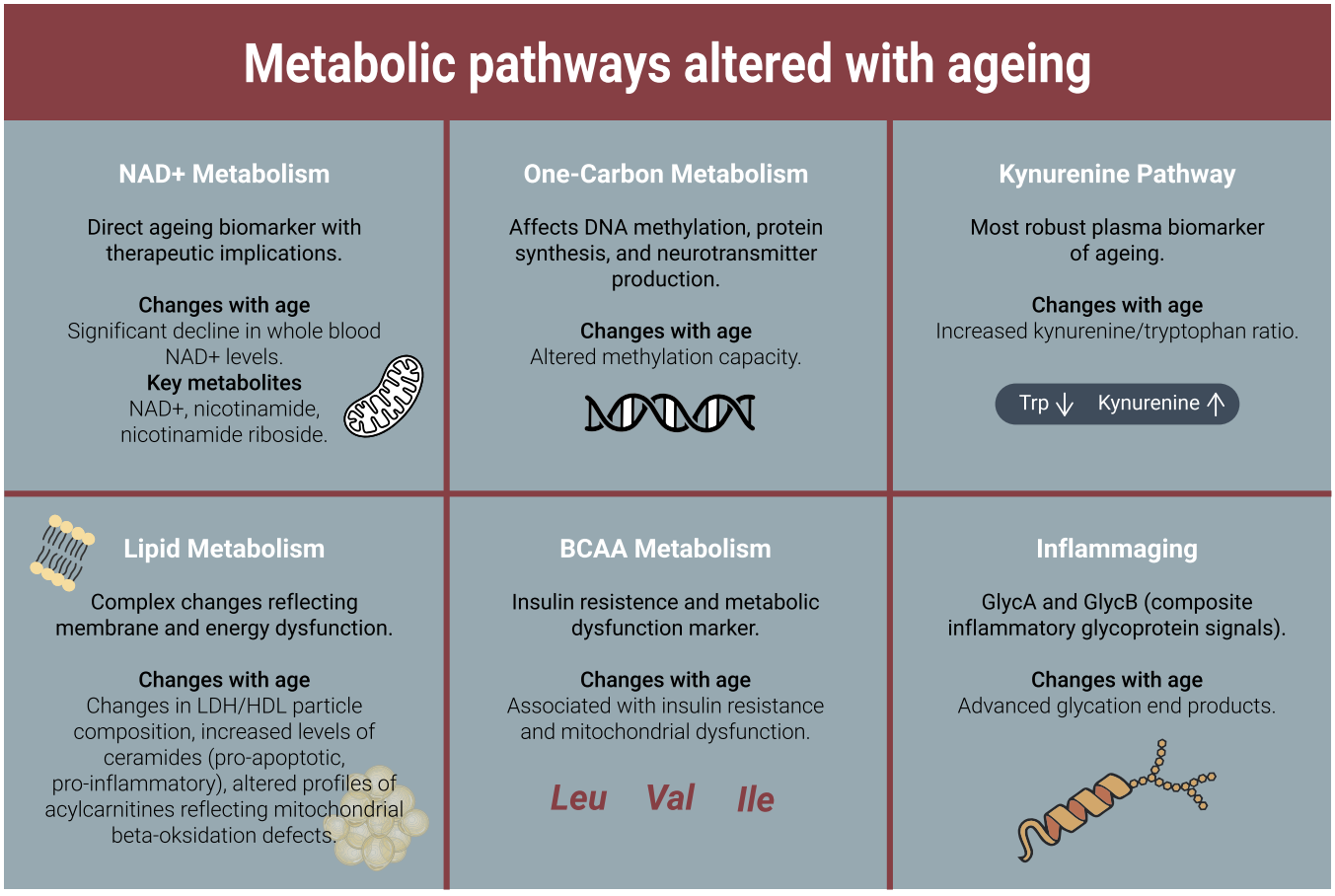

Fig. 2. Major metabolic pathways altered with Ageing. NAD+ metabolic pathways (Clement et al., 2019; Yang et al., 2022); Kynurenine Pathway (Hetherington-Rauth et al., 2024; Solvang et al., 2022); Lipid Metabolism (Jasbi et al., 2023); Branched-Chain Amino Acid (BCAA) Metabolism (Han et al., 2023; Jang et al., 2016); Inflammation Pathways (Inflammaging) (Karas et al., 2022; Lodge et al., 2025); One-Carbon Metabolism (Ducker and Rabinowitz, 2017; Yang and Vousden, 2016). HDL, High-Density Lipoprotein; Ile, Isoleucine; LDL, Low-Density Lipoprotein; Leu, Leucine; Val, Valine.

Recent studies have shown that plasma-based metabolomic clocks can effectively detect biological ageing by showing moderate correlations with chronological age while reflecting underlying metabolic health status (Jansen et al., 2021; Macdonald-Dunlop et al., 2022). Notably, metabolomic measures of ageing show limited overlap with other ageing clocks, such as DNA methylation or proteomic clocks, indicating that the plasma metabolome provides unique biological information about ageing processes (Macdonald-Dunlop et al., 2022). This suggests that incorporating plasma metabolomics into ageing research may provide complementary insights, particularly in identifying metabolic signatures associated with accelerated ageing and age-related disease risk.

In the study that introduced ageotypes, distinct differences in ageing patterns were found between insulin-resistant and insulin-sensitive individuals, with insulin resistance being associated with accelerated changes in inflammation-related plasma biomarkers, suggesting that metabolic health status significantly influences plasma-based ageing profiles (Ahadi et al., 2020).

The plasma metabolome undergoes significant changes with increasing age, which can be attributed to shifts in important metabolic pathways. In a cross-sectional study, 92 age-associated plasma metabolites were identified, the majority of which were lipid species such as phosphatidylcholines and ceramides, most of which increased with age (Moaddel et al., 2024). Markers of oxidative stress (*e.g.* cystine/cysteine ratio) and inflammation (*e.g.* kynurenine/tryptophan ratio) also increased, while DHEAS levels and indicators of arginine bioavailability decreased. These changes suggest age-related impairments in mitochondrial function, vascular health and redox balance. Interestingly, an increase in metabolites associated with a healthy diet and gut microbiota, such as proline-betaine and omega-3 fatty acids, was also observed, suggesting possible compensatory or protective mechanisms in healthy ageing.

In a multi-omics study by Shen *et al.* (2024), it was shown that the functions and risks of age-related diseases change non-linearly over the human lifespan. Plasma metabolomics showed strong correlations with chronological age, with several plasma metabolites showing significant non-linear changes, particularly in midlife and old age. In addition, certain plasma metabolites are associated with an increased risk of age-related diseases, including cardiovascular disease and type 2 diabetes, particularly after the age of 60 (Shen et al., 2024a).

In a large integrative study using blood-derived biomarkers, including metabolic plasma profiles, combining multiple biological clocks into a composite index was shown to improve associations with health outcomes compared to single clocks (Jansen et al., 2021). This approach emphasises the importance of plasma-based metabolomic profiling as part of a multi-omics strategy that provides a more comprehensive assessment of biological ageing and greater predictive value for age-related health risks. In another study, the plasma metabolome of over 11,000 individuals from five Swedish cohorts was profiled, identifying 1629 metabolites with a broad coverage of lipid, amino acid and xenobiotic classes (Ghosh et al., 2024). Approximately 10–20 % of the metabolites showed significant associations with age, indicating metabolic shifts associated with physiological ageing. Among these substances, creatinine, branched-chain amino acids and fatty acids, which are closely related to kidney function, muscle metabolism and dietary intake, were emphasised. The study found that plasma metabolic profiles were largely consistent across populations and stable over time, emphasising their reliability in ageing research. These results offer important insights into metabolic ageing and provide a basis for biomarker discovery and personalised health monitoring.

In a large-scale study of 44,168 people from 12 cohorts, Deelen *et al.* (2019) identified 14 plasma and serum biomarkers that are independently associated with all-cause mortality. The study showed that metabolic biomarkers significantly improve the prediction of 5- and 10-year mortality beyond conventional risk factors, indicating their potential utility for clinical risk stratification, which needs to be further validated (Deelen et al., 2019b).

5.2. Untargeted and targeted approaches to biomarker discovery

Lassen *et al.* (2022) applied untargeted LC-MS metabolomics to plasma samples from 10,133 individuals to investigate metabolic signatures of ageing. Over 200 plasma metabolites, particularly acylcarnitines, steroids and lysophospholipids, showed significant associations with age, reflecting shifts in mitochondrial function, lipid metabolism and endocrine activity. A prediction model based on these metabolites estimated biological age with high accuracy and identified individuals whose metabolomic age exceeded their chronological age, which correlated with poorer health outcomes (Lassen et al., 2023).

A targeted metabolomics study by Jasbi et al. (2023), which compared healthy young adults (21–40 years) with older people (65 + years), demonstrates that the metabolic profiles in plasma change significantly with increasing age. The older group had higher levels of saturated fatty acids such as palmitic and stearic acid and lower levels of metabolites involved in fatty acid oxidation and amino acid metabolism, such as decanoylcarnitine and methylhistamine. These changes indicate impaired mitochondrial function and increased oxidative stress with age and point to potential biomarkers and metabolic pathways that reflect the physiological degradation associated with ageing (Jasbi et al., 2023).

Metabolomics enables the comprehensive profiling of small molecules in biological systems, with blood plasma providing a dynamic and accessible matrix for the study of age-related metabolic changes. The plasma metabolome provides a robust and accessible biomarker landscape for the assessment of biological ageing. Numerous studies have found significant changes in the plasma metabolome with increasing age, particularly in lipid metabolism, redox homeostasis and mitochondrial function. Age-associated metabolites such as phosphatidylcholines, ceramides, acylcarnitines and creatinine have been consistently associated with physiological ageing, while markers such as DHEAS and arginine bioavailability decrease. Large-scale untargeted and targeted metabolomics studies have demonstrated the potential of plasma metabolic profiles to predict biological age and identify individuals at risk of accelerated ageing. Furthermore, the integration of plasma metabolomics into multi-omics approaches increases the predictive accuracy for mortality and healthspan beyond traditional clinical markers. Overall, plasma metabolomics represents a promising avenue for biomarker discovery, personalised assessment of ageing and early detection of age-related health damage.

5.3. Limitations, data normalization, and metabolite stability in ageing metabolomics studies

Ageing metabolomics studies face several critical methodological challenges that must be addressed to ensure robust and reproducible findings. Batch effects from extended analysis periods can confound biological signals and create false age-related trends (Guo et al., 2023; Han and Li, 2022), requiring sophisticated normalization approaches such as CordBat (Guo et al., 2023), WaveICA (Deng et al., 2019), and NormAE (Rong et al., 2020) to preserve genuine biological variation while removing technical artifacts. Metabolite stability during sample collection and storage is particularly crucial, as improper storage conditions (*e.g.,* −20°C vs. −80°C) can significantly alter metabolite profiles (Valo et al., 2022), and pre-analytical procedures including collection tube selection and processing timing critically affect biomarker reliability (Chen et al., 2024; Nishiumi et al., 2018). Additional limitations include the predominance of cross-sectional designs that cannot distinguish ageing from cohort effects (Panyard et al., 2022), substantial inter-individual variability requiring large sample sizes (Kondoh et al., 2020), challenges in metabolite identification and quantification (Tian et al., 2022), and matrix effects that affect measurement accuracy (Martins et al., 2024). To advance the field toward clinical translation,

ageing metabolomics studies must prioritize standardized protocols for sample handling, implement appropriate batch correction methods with quality control samples, validate findings in independent cohorts, and address population heterogeneity through careful study design (Moqri et al., 2024; Singh and Benayoun, 2023). These methodological considerations are essential for realizing the full potential of metabolomics in understanding human ageing biology.

6. Gut microbiome and its role in biological ageing

The human microbiome, comprising trillions of microorganisms inhabiting various body sites, is now recognized as a fundamental component of health. These microbial communities play essential roles in digestion, nutrient absorption, immune system regulation, and protection against pathogens. The gut microbiota, in particular, is deeply involved in metabolic processes, producing key metabolites such as short-chain fatty acids that influence energy balance, lipid metabolism, and inflammation control (Aggarwal et al., 2023; De Vos et al., 2022; Hills Jr. et al., 2022). A balanced microbiome supports homeostasis and helps maintain the integrity of bodily systems. Disruptions in microbial composition, known as dysbiosis, are increasingly linked to a wide range of diseases, including obesity, type 2 diabetes, cardiovascular disease, inflammatory bowel disease, and certain cancers (De Vos et al., 2022; El-Sayed et al., 2021; Hills Jr. et al., 2022; Hou et al., 2022). The microbiome's influence extends beyond the gut, affecting the skin, respiratory, urinary, and reproductive systems, and even modulating the gut-brain axis, which impacts mood and cognitive function (Aggarwal et al., 2023; Hills Jr. et al., 2022). Therapeutic strategies targeting the microbiome, such as probiotics, prebiotics, dietary interventions, and faecal microbiota transplantation, are being explored to restore microbial balance and treat disease (Bidell et al., 2022; Hou et al., 2022). However, defining a universally "healthy" microbiome remains challenging due to individual variability and the complex interplay between microbes and host factors (Joos et al., 2025; Van Hul et al., 2024).

The study of the microbiome has rapidly evolved, driven by advances in sequencing technologies, computational tools, and multi-omics integration. 16S rRNA gene amplicon sequencing and shotgun metagenomics allow for comprehensive taxonomic and functional profiling of microbial communities (Bharti and Grimm, 2021; Jovel et al., 2016; Knight et al., 2018; Pérez-Cobas et al., 2020; Ranjan et al., 2016). High-throughput sequencing (HTS) technologies, including both short-read and long-read platforms, have enabled unprecedented resolution in microbiome analysis, while metatranscriptomics, metaproteomics, and metabolomics provide insights into microbial function and activity (Bauermeister et al., 2022; Zhang et al., 2019). Computational and statistical methods (further discussed in Chapter *8. Computational modelling of biological age*), including machine learning and network analysis, are essential for interpreting the complex datasets generated (Marcos-Zambrano et al., 2021; Matchado et al., 2021; Moreno-Indias et al., 2021; Namkung, 2020). Recent innovations such as single-cell genomics, spatial mapping, and integrative multi-omics approaches are pushing the boundaries of what can be learned about microbial communities and their interactions with hosts and environments (Duan et al., 2025; Lloréns-Rico et al., 2022; Zheng et al., 2022).

6.1. Age-related changes in gut microbiome diversity

Comprehensive reviews of human studies reveal that the gut microbiome undergoes notable shifts throughout the lifespan. Healthy ageing appears to be linked to increased diversity and uniqueness in the gut microbiome, especially in individuals over 80. This uniqueness is associated with beneficial metabolic outputs and predicts better survival, while a lack of diversity or dominance of certain bacteria (*e.g. Bacteroides*) is linked to poorer outcomes (Wilmanski et al., 2021). The increase in microbiome uniqueness with age occurs in both males and females but is interestingly 50 % more pronounced in females. Individuals with increasingly unique gut microbiomes as they age also show specific changes in their plasma metabolome. This appears to be true particularly in microbial derived amino acid derivatives, for example in plasma phenylalanine/tyrosine and tryptophan metabolites, which have been implicated in longevity. Phenylacetylglutamine and p-cresol sulfate are such metabolites that demonstrated some of the strongest associations with gut microbial uniqueness. As have indoles, gut microbiome degradation products of tryptophan, which have been shown to increase health span and extend survival in a number of animal models (Wilmanski et al., 2021).

Age-related changes in the microbiome and various biological markers in the body can best be determined by different omic approaches. In their study on a longitudinal human cohort of 108 participants aged between 25 and 75 years, Shen *et al.* (2024) used several omic approaches such as transcriptomics, proteomics and metabolomics in addition to metagenomic sequencing of the microbiome. Interestingly, they found that age-related molecular changes and changes in the microbiome over the human lifespan are not linear, but occur in two waves around the ages of 44 and 60, which are associated with several dysregulated functional modules (Shen et al., 2024a). While the transition around age 60 is marked by significant shifts in immune regulation, including increased chronic inflammation, altered immune cell composition, and dysregulation of cytokine signalling pathways, the wave around age 44 is associated with dysregulation in cardiovascular function, lipid metabolism, and alcohol metabolism.

6.2. Microbiome-based ageing clocks

Microbial profiles across various body sites can serve as reliable predictors of chronological age (Martino et al., 2022). Ageing clocks, which estimate biological age based on molecular and physiological markers such as DNA methylation, blood biomarkers, and increasingly, microbiome composition, have gained importance in recent years. Biological age, reflecting cumulative molecular damage and physiological decline, provides a more accurate measure of health status and disease risk (Mathur et al., 2024). Recent advances in genome sequencing have enabled the development of epigenetic clocks based on DNA methylation patterns (McCartney et al., 2021), further enhancing our understanding of ageing mechanisms.

Human microbiome-based ageing clocks developed using microbial taxonomy, diversity metrics and functional pathways hold promise for estimating biological age. These clocks are modulated by microbial metabolites and clock gene regulation, which can influence ageing trajectories. For instance, Chen et al. (2022) used machine learning to construct an ageing clock using metagenomic data from 4478 faecal samples (Chen et al., 2022). They identified several biomarkers such as specific species abundance and metabolic pathways enrichment associated with ageing-related diseases and nutrient utilisation patterns, integrating these features into a predictive model. Galkin et al. (2020) developed an ageing clock based on more than 4000 metagenomic profiles of people aged 18–90 years using a deep neural network (DNN) model. They showed that most species either increase or decrease the estimated age, while some species are not consistently associated with changes in predicted age (Galkin et al., 2020). Ahadi et al. (2020), who studied a cohort of 106 prediabetic and healthy individuals aged 29–75 years, were able to positively correlate the abundance of certain groups or genera, such as *Clostridium* cluster IV and the genus *Blautia*, with age, which is consistent with the prediction of the DNN model (Ahadi et al., 2020).

Ahadi et al. (2020) classified individuals into four ageotypes on the basis of the distinct types of molecular pathways that change over time. Although the term ageotype has not yet been applied to microbiomes or microbiota, different microbiome profiles could potentially be referred to as microbiome ageotypes. Several studies have highlighted the development of biological ageing clocks (Chen et al., 2022; Galkin et al., 2020; Wang et al., 2024). In this context, not only can biological age be

predicted, but microbiome ageotypes could also be inferred from gut microbiome metagenomic data using machine learning models, particularly when multi-omics data are integrated. However, further research is needed to determine whether modulating gut microbiota can effectively influence these ageotypes in humans.

Reicher et al. (2024) pointed to gender-specific differences in ageing patterns using data from a longitudinal study of a cohort of 10,000 healthy individuals aged 40–70 years, who were followed up every two years over a total period of 25 years. In their study, the biological age score based on blood lipids correlated significantly with the gut microbiome in the male but not in the female study group (Reicher et al., 2024).

Accelerated ageing, a condition where biological age exceeds chronological age, has also been linked to the gut microbiota. In a study on Asian women of reproductive age (S-PRESTO cohort from Singapore), biological ageing was investigated together with clinical, nutritional, lipidomic, and genetic factors (L. Chen et al., 2024). Analysis of 630 participants from a multi-ethnic cohort aged 18–45 years revealed that certain taxa such as *Erysipelotrichaceae* UCG-003 and *Bacteroides vulgatus* were inversely associated with PhenoAgeAccel, a marker of accelerated ageing, and some blood biomarkers.

A broader investigation involving 18,340 individuals across 24 cohorts that included GWAS, further supported a genetic correlation between gut microbiota, intrinsic epigenetic age acceleration, and frailty (Yan et al., 2025). In this study, certain bacterial genera were found to be associated with the protective effects, for example *Prevotella* in the case of accelerated ageing, and *Flavonifracter* and *Victivallis* in the case of frailty.

6.3. Gut microbiota, disease susceptibility, and ageing phenotypes

Age-related changes in the gut microbiome can compromise immune function and increase susceptibility to diseases. Lu et al. (2025) used Mendelian Randomization (MR), Linkage Disequilibrium Score Regression (LDSC), and machine learning to investigate associations between gut microbiome, ageing indicators (telomere length, frailty index, facial ageing), and 14 age-related diseases across diverse global cohorts (Lu et al., 2025). The data sets of the gut microbiota of several cohorts from Europe, Asia, Africa and America with a total of 18,340 participants were included in the analysis. Their findings highlighted specific bacterial taxa as potential biomarkers for disease risk and ageing.

Sarcopenia, characterized by the progressive loss of muscle mass and strength, is another ageing-related condition linked to gut microbiota. A study using data from over 6000 participants (40.20 ± 13.78 years old) in the U.S. National Health and Nutrition Examination Survey (2007–2018) examined the relationship between the Dietary Index for Gut Microbiota (DI-GM), novel metric assessing diet quality in relation to gut health, and sarcopenia, incorporating biological ageing markers such as PhenoAge, the Klemera-Doubal Method (KDM), and Homeostatic Dysregulation (Zhang et al., 2025). The DI-GM calculated from the data obtained by nutrition survey using recall interviews, was significantly associated with reduced sarcopenia risk, with biological age indicators mediating this relationship (Kase et al., 2024).

Gut microbiota may influence ageing clocks through metabolite production and clock gene modulation. Recent Mendelian randomization studies have identified causal links between specific bacterial taxa and epigenetic age acceleration (EAA). Xu et al. (2024) analysed data from 18,340 participants using four epigenetic clocks (IEAA, HannumAA, GrimAA, PhenoAA), revealing that taxa such as *Acidaminococcaceae* and *Clostridiaceae*1, families associated with production of short-chain fatty acids, were associated with reduced EAA, while *Holdemania*_unclassified was linked to increased EAA (H. Xu et al., 2024).

Similarly, Ye et al. (2024) reported a causal relationship between *Streptococcus* abundance and accelerated biological ageing (PhenoAgeAccel and BioageAccel predictors), using data from the MiBioGen consortium and GWAS (Ye et al., 2024). Given that *Streptococcus* genus includes opportunistic pathogens, its dysbiosis may contribute to inflammation, oxidative stress, and age-related diseases.

Emerging evidence also suggests that gut microbiota mediates the relationship between diet and biological ageing. Zhang et al. (2024) studied 8288 participants aged 30–79 years, from less-developed regions in China (CMEC). The main findings were that adherence to healthy dietary patterns (plant-based diet index - PDI, healthy PDI - hPDI, HDS, dietary approaches to stop hypertension - DASH and alternative Mediterranean diets - aMED), especially DASH and aMED, was inversely associated with biological age acceleration (KDM-AA) while unhealthy PDI (uPDI) was positively associated with KDM-AA (Zhang et al., 2024). The results also indicated that the decreased abundance of the *Synergistetes* phylum and its component genus *Pyramidobacter* may mediate the negative associations between plant-based diets and KDM-AA.

A similar approach (16S rDNA sequencing of faecal microbiome, determination of KDM-BA and Dietary Screener Questionnaire) was used in the 10,000 Families Study, involving 117 individuals aged 19–91 (Sharma et al., 2024). Accelerated KDM-BA was positively associated with *Streptococcus* and negatively associated with *Subdoligranulum*, unclassified *Bacteroidetes*, and *Burkholderiales*, suggesting that reduced abundance of these taxa may contribute to biological ageing.

Current research underscores a strong link between gut microbiome composition and biological ageing. Alterations in microbial diversity and function are associated with ageing clocks, physical health, and disease risk. While epigenetic ageing, measured by DNA methylation, is tied to mortality and physiological stress, the direct role of the microbiome in modulating epigenetic ageing warrants further exploration. Nonetheless, these findings highlight the potential of microbiome-targeted interventions to promote healthy ageing and longevity.

Boxmary Box 4: Gut microbiome and biological ageing

The gut microbiome is a key regulator of metabolism, immunity, and systemic health, with imbalances linked to numerous chronic diseases. Advances in sequencing, multi-omics, and computational tools have revolutionized microbiome research, enabling detailed insights into its role in ageing.

Age-related changes in gut microbiome diversity: Healthy ageing is linked to increased gut microbiome diversity and individuality, particularly in those over 80. Unique microbial profiles correlate with beneficial metabolite production, such as indoles, which are associated with longevity. Multi-omics studies reveal that microbiome and molecular changes occur in two major waves around ages 44 and 60.

Microbiome-based ageing clocks: Gut microbiome composition can accurately predict chronological and biological age using machine learning models. Microbiome-based ageing clocks reveal associations between specific taxa, metabolic pathways, and ageing acceleration or protection. Sex-specific patterns exist, with certain microbiome–biological age correlations more pronounced in men than in women.

Gut microbiota, disease susceptibility, and ageing phenotypes: Age-related microbiome shifts contribute to disease susceptibility, including sarcopenia and frailty, through immune and metabolic pathways. Specific bacterial taxa influence epigenetic age acceleration, linking microbiome composition to molecular ageing markers. Healthy dietary patterns may slow biological ageing partly by promoting beneficial gut microbial profiles.

7. Effects of exercise on biological ageing

Exercise is a powerful modifier of both physical decline and non-communicable disease state, cross-cutting key variables necessary to measure when refining any biological clock(s), and critical to consider when guiding the development of effective programme interventions. Performing adequate and regular physical activity is promoted by global health organisations due to its proven ability to reduce harmful disease

states, including its ability to effectively stimulate longer disease-free survival from *e.g.* cancer (Courneya et al., 2025). The burgeoning evidence supporting how physical activity acts as an environmental stressor is present in studies which report discernible impacts on epigenetic modifications, including: histone modifications, DNA methylation, RNA methylation, and non-coding RNA, among others (Zheng et al., 2025) especially for the heart, brain and muscle. Exercise positively impacts methylation, inflammation, and metabolism by promoting beneficial epigenetic changes that regulate genes involved in these processes. Exercise can reduce chronic inflammation and improve metabolic health by altering DNA methylation patterns, improving insulin sensitivity, and regulating genes involved in fat storage, muscle repair, and energy use (Etayo-Urtasun et al., 2024; Hawley et al., 2014; Mallett, 2025; Zheng et al., 2025).

A growing body of evidence shows that physical activity and exercise induce molecular and physiological adaptations in skeletal muscle that help preserve health and mitigate chronic disease risk. These adaptations arise from complex interactions between genetic and environmental influences and are primarily mediated by exercise-induced transcriptional responses (Mallett, 2025). Both endurance and resistance training modulate key metabolic and regulatory pathways, leading to structural and functional remodelling of skeletal muscle. The most robust and direct evidence that one's fitness status can slow/augment biological ageing is provided by a handful of studies over the past five years. For example, Tucker and Bates (2024), in their large, US sample (N = 4814), found that regular 90 min of weekly strength training was strongly associated with longer telomeres, equivalent to 3.9 years less biological ageing, even after adjusting for confounders (Tucker and Bates, 2024). Similarly, in older Mexican adults (N = 323), better physical performance was associated with significantly longer telomeres (Martínez-Ezquerro et al., 2024). They found that the odds of low physical function increased substantially with each 1 kb of telomere attrition. There were a series of studies from a Finnish working group who also found positive benefits of physical activity on cardiovascular risk factors and genetic bases of all-cause mortality (Herranen et al., 2025; Joensuu et al., 2025). Different measurement tools have been able to provide similar findings. In Singapore's oldest-old cohort study, comprising 433 persons, (median age ~88 y), better physical function was associated with lower DNAm measured via GrimAge2, which was deemed the most sensitive epigenetic clock to monitor physical function, showing clear links between better fitness and slower epigenetic ageing (Tay et al., 2025). FitAge was predicted considering the results of six motor-functional fitness tests (Four Square Step Test, Timed Up and Go, 10-Meter Timed Walk, 6-Minute Walk Test, maximum voluntary isometric contraction of the dominant quadriceps, handgrip test) as predictors which outperformed BMI in predicting biological ageing trajectories and health risk (Manca et al., 2024). Specifically, FitAge could differentiate decelerated vs. accelerated agers across immune, body composition, and inflammatory domains. Whereas previous epigenetic clocks were developed using blood-derived data, a recent study has introduced the CheekAge clock, a next-generation model optimized for predicting biological age from cheek swabs, which are non-invasive, and simpler to obtain. Based on the CheekAge clock, epigenetic ageing has been linked to self-reported behavioural factors like exercise and diet (Shokhirev et al., 2024). However, the operationalization of exercise in these analyses, whether reflecting its volume, intensity, or total load, remains unclear. Since self-reported behaviours may not fully capture the physiological adaptations induced by habitual physical activity, physical fitness likely represents a more integrative and objective indicator of functional biological ageing. Thus, combining fitness-based measures with self-reported behavioural data and omics-derived biomarkers may therefore enhance the precision of multi-domain biological ageing models.

7.1. Mechanistic, multi-omic, or biomarker-focused studies supporting fitness–ageing links

Endurance athletes and calorie-restricted adults have shown lower biological age across six omics layers compared to sedentary peers in work communicated by Fiorito and colleagues (Fiorito et al., 2025). These exercise-induced reductions were especially strong in epigenetic and transcriptomic markers from colon tissue. In general, there is growing evidence that healthier microbiomes linked to anti-inflammatory species are associated with slower epigenetic ageing. In terms of an exercise context, and amongst physically active older individuals, gut microbiome diversity has been inversely associated with epigenetic ageing and overall physical fitness (Torma et al., 2024), with more work needed to confirm these initial findings. Ageing biomarkers, like GDF-15 may serve as an accessible proxy of physical decline and epigenetic ageing since it has been correlated with lower grip strength, worsening lung function, and higher epigenetic age (participant age range: 20–88 y) (Torrens-Mas et al., 2025). Considering these relationships, some investigations have designed longitudinal designs with exercise interventions to better understand possible causal mechanisms between exercise prescription and the ageing response. For example, a one-year supplement trial consisting of performing very minimal physical activity durations of one 10-min walk performed daily, showed improved physical performance and reduced epigenetic age in its participants (Carreras-Gallo et al., 2025), providing a possible 'floor response' to positive health outcomes (*i.e.* smallest physical activity dose which can still provide physiologically relevant improvements). Likewise, in a 2.7-year follow-up study in older adults, researchers found that vitamin D receptor genotypes (*e.g. Apa*I) influenced declines in muscle strength (Krasniqi et al., 2025). The existence of a genetics-based vitamin D pathway modulating age-related fitness loss highlights the necessity to better isolate interactions between fitness, ageing, and genomics.

7.2. DNA methylation-based ageing (epigenetic clocks)

Higher fitness and exercise habits are consistently linked to *younger biological age* as measured by epigenetic clocks, with newer clocks like DNAmFitAge being more sensitive to fitness-related differences. Exercise can induce changes in DNA methylation patterns, which are an epigenetic mechanism that controls gene expression. Importantly, recent research has identified epigenetic regulation as a central mechanism underlying these exercise adaptations. Exercise triggers signalling cascades that modify myofiber metabolism and contractile properties through dynamic changes in DNA methylation, histone modification, and non-coding RNA activity. These epigenetic modifications play a pivotal role in orchestrating gene expression patterns that contribute to the beneficial effects of exercise and training-induced plasticity (Mallett, 2025). Specifically, muscle contraction triggers several cellular processes, including Ca^{2+} release and uptake, changes in AMP/ATP ratios that activate AMPK, and increased oxidative metabolism that generates reactive oxygen species (ROS). These processes influence the availability of S-adenosylmethionine (SAM), a methyl donor, thereby affecting DNA methylation patterns. Thus, oxidative stress and calcium signalling are key regulators of exercise-induced DNA methylation (Williams et al., 2025). Several studies reinforce the observation that higher cardiorespiratory fitness (CRF) and regular physical activity are associated with a slowing of epigenetic ageing, especially as measured by second-generation clocks like GrimAge, PhenoAge, and FitAge. Specifically, Kawamura et al., (2024) report that VO_2 at ventilatory threshold (and peak) are negatively associated with GrimAgeAccel, independent of smoking or alcohol consumption, although they note that CRF demonstrated a smaller contribution than other factors (*e.g.* smoking, triglycerides, carbs) (Kawamura et al., 2024b). Promisingly, da Silva Rodrigues and colleagues (2024) performed an 8 week combined aerobic and strength training regime which significantly reduced the

epigenetic age in women who displayed initially high age acceleration (Da Silva Rodrigues et al., 2024), and Jokai et al. (2023) found that higher fitness (measured via: VO_2max, grip strength, vertical jump), was linked to younger biological age by DNAmFitAge (Jokai et al., 2023). This tool was also able to better distinguish high- vs. low-fit individuals than GrimAge/PhenoAge. Interestingly, Seki and colleagues (2023) found that DNAm-based telomere length (DNAmTL), but not traditional telomere assays, significantly correlated with CRF and other fitness metrics, suggesting that DNA methylation may better reflect fitness-related ageing mechanisms than direct telomere measures, *per se* (Seki et al., 2023). Finally, Aczel et al., (2023) found that although physical fitness was related to lower PhenoAge, this was only observed in men, and associated with higher circulating levels of Klotho, a longevity protein affected by methylation (Aczel et al., 2023).

7.3. Metabolomics, mitochondrial health, and fitness

Metabolic pathways tied to mitochondrial function, inflammation, and muscle performance are crucial mediators between physical activity and biological ageing—supporting metabolomics as a valuable ageing biomarker domain. Obesity is also well-known to contribute to chronic low-grade inflammation via increased secretion of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β from macrophages infiltrating adipose tissue (Liu et al., 2020). Although acute exercise is accompanied by a temporary inflammatory response, over time, exercise can lead to changes in DNA methylation that reduce the body's pro-inflammatory state. Regular moderate-intensity exercise is known to reduce chronic inflammation by reducing the expression ofpro-inflammatory cytokines like *IL-6* and *TNFα* (Zheng et al., 2025). Additionally, strength training is recognized as an effective intervention for reducing the risk of obesity and metabolic diseases. It promotes several beneficial changes in adipose tissue, such as lowering adipocyte size, modulating pro-inflammatory adipokines, decreasing reactive oxygen species and inflammation, and increasing vasculature, *i.e.,* processes that are often dysregulated in obesity (Ostaiza-Cardenas et al., 2025). There were two large metabolomic studies, each highlighting the systemic biological imprint of fitness across the lifespan. The first, from Yao et al. (2024) compiled four metabolic scores (body composition, mental-physical performance – neuro-cognitive tests), muscle strength, physical activity – questionnaire), which correlated with healthy ageing and lower disease risk (Yao et al., 2024). These scores, developed in the elderly (but validated in young adults), predict long-term outcomes including CVD and cognitive decline, and suggest that one's early-life metabolic fitness predicts later biological ageing. The second study, by Youssef et al. (2023) found that High Intensity Intermittent Training (HIIT) in obese older adults led to favourable changes in 51 serum metabolites (Youssef et al., 2023). Metabolites like 2-oxoglutaric acid (linked to insulin sensitivity) and PCae (related to grip strength) may serve as biomarkers of fitness-driven ageing resilience. A recent study by Ostaiza-Cardenas et al. (2025) showed that activities, like HIIT and training that aims to improve both muscle strength and cardiorespiratory fitness concurrently, induced epigenetic modifications, improving metabolic function, mitochondrial biogenesis, and insulin sensitivity (Ostaiza-Cardenas et al., 2025). We reiterate that based on the available evidence, exercise improves metabolic health by positively impacting glucose homeostasis and insulin sensitivity (Zheng et al., 2025). Exercise-related epigenetic adaptations within skeletal muscle involve key regulatory genes, including PGC-1α (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha) and PPARγ (Peroxisome Proliferator-Activated Receptor Gamma), which play central roles in mitochondrial biogenesis, oxidative metabolism, and lipid utilization. Exercise-related changes in methylation patterns have also been observed in the ASC (Apoptosis-Associated Speck-Like Protein Containing a CARD) gene, suggesting enhanced regulation of inflammatory pathways. Moreover, epigenetic biomarkers are increasingly recognized as valuable indicators of metabolic responsiveness to behavioural interventions. Specific DNA methylation signatures in genes like IGF2 (Insulin-Like Growth Factor 2) and leptin have been proposed to be potential predictors of individual responses to dietary and physical activity programs (Ostaiza-Cardenas et al., 2025). Taken together, the importance of adequate fitness and its impact on metabolomics do affect biomarkers of ageing, whether across the lifespan, or as part of an exercise intervention.

7.4. Functional measures and healthy ageing

Objective measures like grip strength, forced vital capacity (FVC), mobility, and vascular health remain robust indicators of ageing phenotype, and are tightly connected to fitness and other life choices such that we can comfortably assert that functional capacity is a tangible proxy of biological age. Recently, Obisesan et al. (2024) found that in adults aged 75 + , high coronary artery calcium (CAC) was linked with greater impairment across ageing domains (*e.g.,* cognition, handgrip, FVC), whilst CAC= 0 was associated with healthier ageing, highlighting vascular ageing as a systemic marker of ageing biology (Obisesan et al., 2024). In older Chinese adults, mobility, handgrip/pulmonary, and muscle strength were identified as three latent constructs of physical function that offer a validated framework for assessing and targeting healthy ageing interventions likely since they directly affect daily physical function (Jiang et al., 2023).

7.5. Telomere length and fitness: mixed evidence

In general, fitness may not consistently slow telomere shortening, as measured by standard assays, but methylation-derived telomere length (DNAmTL) does provide some more sensitive insight (Seki et al., 2023). In their work, traditional telomere length (TL) was not associated with fitness, but DNAmTL was found to do so. Also, PhenoAge and GrimAgeAccel inversely correlated with TL, suggesting an indirect link between fitness and telomere biology, mediated via epigenetic regulation. Functional fitness as a surrogate for biological age is regularly researched in the literature. For example, Toyoshima et al. (2022) introduced a physical fitness age based on grip strength, balance, and walking speed (Toyoshima et al., 2022). This measure predicted sarcopenia more accurately than chronological age, and was associated with modifiable risk factors (diabetes, obesity, malnutrition), whilst Foroni et al. (2022) found that amongst older individuals aged 80 + , high levels of FGF23 (a biomarker linked to mineral metabolism) correlated with lower muscle strength, poorer aerobic capacity, and more falls, indicating poorer musculoskeletal ageing and reduced functional capacity (Foroni et al., 2022). In older obese adults, improvements in grip strength and gait speed over 12 weeks were significantly associated with reductions in DNA methylation age (Hannum and PhenoAge clocks), also suggesting a reversal or ameliorisation of biological age via physical function improvement (Petersen et al., 2021). Mechanisms behind these observations may include molecular pathways linking physical fitness and ageing such that aerobic fitness indeed positively correlates with telomere length, as mentioned in the sections above, and this phenomenon has been seen in programmes comprised of six weeks of sprint interval training (SIT), found to alter the expression of over 100 microRNAs, especially those regulating immune function and lipid metabolism (Kumar Dev et al., 2021). Muscle-specific epigenetic clocks have weaker associations with physical fitness compared to blood-based clocks (Sillanpää et al., 2021), suggesting current epigenetic models may insufficiently capture muscle-related ageing processes.

A study investigating telomere length and frailty found that amongst > 440,000 UK Biobank participants, shorter leucocyte telomere length (LTL) was significantly associated with frailty indicators like low grip strength, slow walking, and exhaustion, even after adjusting for age and other social determinants (Bountziouka et al., 2022). Whole-system biological ageing and fitness studies have also been used across a wide variety of systems, including the liver, immune and kidney, where

researchers used physical fitness as a composite biomarker. One study from Nie et al. (2022) used multi-omics (including fitness tests) to estimate organ/system-specific biological ages. Ageing rates varied widely such that faster ageing in key systems correlated with poorer survival outcomes (Nie et al., 2022). Chan et al. (2021) had earlier developed a composite biomarker for age using 72 physiological measures. Key contributors to the model included grip strength and lung/kidney function. The composite system outperformed chronological age in predicting mortality and hospitalizations, confirming fitness as a key component when assessing biological age and clinical health outcomes (Chan et al., 2021). Perhaps, this is because of longstanding genetic information. In a fascinating study, Sarnowski et al., (2021) identified rare and ancestry-specific genetic variants linked to handgrip strength. Many of these genes were found to be involved in brain and muscle function, reinforcing the genetic basis of strength and its relevance for healthy biological ageing trajectories (Sarnowski et al., 2021).

To sum, physical fitness, especially aerobic capacity and muscular strength, is: *(a)* inversely associated with biological age acceleration (BAA) and DNA methylation clocks (GrimAge, PhenoAge, FitAge), *(b)* linked to longer telomeres and favourable oxidative stress profiles, *(c)* predictive of frailty, mortality, cognitive decline, and functional limitations, even more so than chronological age, or some composite biological age scores (see Table 3 for an overview of key domains and evidence). Upon literature reflection, emerging themes emphasise that physical performance-based metrics (*i.e.,* grip strength, gait speed, or functional tasks) may outperform multi-system biological ageing indices in predicting adverse health outcomes. Also, longitudinal data supports the assertion that both habitual physical activity and structured fitness programmes performed in adulthood/midlife are associated with a slowing or modifying of biological ageing trajectories. Finally, body composition, especially lower adiposity levels, appear to directly mediate the relationship between fitness and various ageing biomarkers (*e.g.,* oxidative stress, telomere length, epigenetic age). For a visual overview of the mechanisms illustrating how exercise induces epigenetic modifications in skeletal muscle, brain and heart, we would like to refer readers to Fig. 1 from a recent paper by Zheng et al. (2025), and to Table 1 for a summary of studies on the relationship between physical activity and epigenetics by Williams et al. (2025).

Table 3
Fitness and biological ageing. Key domains and evidence.

Domain	Fitness indicator	Ageing biomarker (s)	Main finding
Epigenetic Clocks	VO_2max, strength, CRF	GrimAge, PhenoAge, FitAge, DNAmTL	Higher fitness = lower epigenetic age, especially via newer clocks
Telomere Length	Strength, SPPB, training dose	TL (qPCR), DNAmTL	Mixed results; stronger links via DNAmTL than traditional TL
Metabolomics & Mitochondria	Walking speed, HIIT, grip strength	Citric acid, 2-oxo-glutarate, PCae, GDF-15	Fitness alters bioenergetic and anti-inflammatory metabolic pathways
Functional Ageing	Grip strength, gait speed, IADL	Composite frailty, vascular health, CAC	Performance metrics robustly predict ageing phenotype and adverse outcomes
Multi-Omic Ageing	Elite fitness vs sedentary	Integrated omics (epigenome, transcriptome, *etc.*)	Athletes show slower ageing across systems, especially epigenetic markers
Fitness-Based Biological Age	FitAge, Physical Fitness Age	FitAge, composite performance models	Outperforms BMI/ chronological age in ageing prediction
Interventions	Aerobic + strength, light walking	DNAmAge, physical function	Even low-dose programs can reduce biological age markers
Genetic Moderators	VDR genotypes, rare strength SNPs	Muscle strength decline, functional trajectories	Genetic variation modulates age-related fitness decline

Boxmary Box 5: Physical fitness and ageing

A growing body of research supports the role of physical fitness, particularly aerobic capacity, muscular strength, and functional performance, in decelerating biological ageing. Across 34 studies, the following patterns consistently emerge:

Epigenetic age: Higher fitness levels (VO_2max, strength, gait speed) are associated with lower biological age as measured by second-generation DNA methylation clocks like **GrimAge**, **PhenoAge**, and **FitAge**. Fitness-related clocks (*e.g.,* FitAge) show greater sensitivity to physical status than traditional clocks.

Telomere biology: While associations between standard telomere length and fitness are mixed, **DNAm-derived telomere length** (DNAmTL) have demonstrated stronger links, suggesting that epigenetic regulation better captures the ageing–fitness interface.

Metabolomic & mitochondrial health: Fitness improves metabolic profiles and mitochondrial markers tied to ageing resilience. Metabolites linked to inflammation and energy balance mediate these relationships.

Functional fitness as a biological ageing proxy: Performance-based measures such as grip strength, walking speed, and physical function consistently outperform chronological age, and even some multi-omic models, in predicting ageing-related outcomes like frailty, disability, and mortality.

Multi-omic and mechanistic insights: Active individuals show favourable ageing profiles across omics domains (epigenome, transcriptome, microbiome, metabolome), with some fitness interventions reversing age-related biological markers.

Targeted interventions: Even short-term or low-dose exercise programs yield measurable reductions in biological age, particularly when combined with dietary or supplementation strategies.

Genetic moderators: Polymorphisms in vitamin D pathways and muscle-related genes influence individual responses to ageing and fitness, highlighting gene–environment interactions.

8. The role of nutrition in regulating biological age

The idea that biological and chronological age can diverge gained prominence with Horvath's epigenetic clock (Horvath, 2013), which predicted age from > 350 CpG sites and linked deviations to disease risk (Mascarelli, 2013). Building on this, refined biomarkers like PhenoAge, GrimAge, and DunedinPACE capture molecular damage, immune decline, and metabolic drift, processes notably influenced by diet (Chan et al., 2025; Vaidya et al., 2023). Given that nutrition influences key hallmarks of ageing, the epigenome may act as a molecular record of long-term dietary patterns.

8.1. Mechanistic links between nutrients and the epigenome

Diet modulates the epigenome via several mechanistic pathways, including one-carbon (1-C) metabolism, regulation of energy and macronutrient intake (*e.g.,* caloric or intermittent restriction), redox balance and inflammatory tone, and the provision of key metabolic co-substrates that act as donors or cofactors for epigenetic enzymes.

8.1.1. One-carbon (1-C) metabolism

One-carbon (1-C) metabolism connects nutrient intake to epigenetic regulation, influencing gene expression, ageing, and disease risk. It provides methyl groups for DNA methylation via key nutrients – folate (vitamin B9), vitamins B2 (riboflavin), B6, B12, and methionine. 5-methyltetrahydrofolate donates a methyl group to homocysteine (via B12-dependent methionine synthase) to form methionine, which is then converted to SAM, the universal methyl donor. Vitamin B6 supports homocysteine clearance, and B2 activates folate. Alcohol disrupts folate metabolism, impairing methylation.

Betaine and its precursor choline serve as backup methyl donors

when folate/B12 are low, while zinc, magnesium, serine, and glycine support methylation enzymes (Dhillon et al., 2024). Vitamin D also influences methylation, particularly in immune-related genes. Recent data link higher dietary creatine with reduced epigenetic mortality risk (Ostojic and Kavecan, 2025).

These nutrients help maintain methylation stability across ageing, neurodevelopment, and cancer. Their deficiency can lead to epigenetic drift (Caffrey et al., 2023). In > 3000 adults, high folate and low homocysteine were tied to younger epigenetic age (Bozack et al., 2025). Yet, high-dose folic acid + B12 trials show mixed results and potential safety concerns (García-García et al., 2024a). A food-first approach, greens and mushrooms, legumes, whole grains, nuts and seeds, eggs, seafood, supports methylation without disrupting 1-C balance.

8.1.2. Energy and macronutrient intake regulation

Excess calories, especially from protein and carbohydrates, activate mTOR and insulin/IGF-1 pathways, promoting growth but inhibiting autophagy and sirtuins, which are essential for mitochondrial and genomic stability. Caloric restriction (≥10 %), amino acid limitation (*e.g.,* methionine, leucine), ketogenic diets, and intermittent fasting counteract these effects and slow ageing (Vasim et al., 2022). In the CALERIE Phase 2 trial, ~12 % calorie reduction over two years slowed DunedinPACE ageing by 2–3 %, with genome-wide shifts toward youthful methylation (Waziry et al., 2023).

Fasting and methylation-supportive diets may even reverse ageing markers in small studies (Perlmutter et al., 2024). Caloric restriction mimetics like β-hydroxybutyrate (βHB) replicate these effects via histone deacetylases (HDAC) inhibition (Liu et al., 2022), and ketone esters benefit brain and vascular health. Pentadecanoic acid (C15:0) mimics rapamycin's effects and improves inflammation markers (Robinson et al., 2024; Venn-Watson, 2024). Spermidine (*e.g.,* wheat germ, natto, aged cheese, mushrooms) also enhances autophagy and cognitive function (Schwarz et al., 2022).

While protein supports muscle, excess (especially from methionine-rich animal foods) may accelerate ageing (Guo et al., 2022). Methionine restriction extends lifespan and rejuvenates DNA methylation in animals (Hernández-Arciga et al., 2025), and plant-based or pescatarian diets are linked to younger DNAm age in humans (Dwaraka et al., 2024). Balancing amino acids while moderating methionine (*e.g.,* via legumes, fish, dairy) may protect both muscle and epigenetic health (Hu, 2024).

High sugar intake raises IGF-1 and oxidative stress, key drivers of ageing. Each 10 g of added sugar correlated with ~0.3 years older PhenoAge in midlife women (Chiu et al., 2024). Reducing sugars and refined carbs in favour of fibre-rich, low-glycaemic foods supports healthier ageing (Bordoni et al., 2024).

8.1.3. Cellular redox and inflammatory tone

Polyphenols (*e.g.,* epigallocatechin-3-gallate – EGCG, berries, herbs, extra virgin olive oil, curcumin, resveratrol), omega-3s (EPA, DHA), and isothiocyanates (*e.g.,* sulforaphane) have potent antioxidant and anti-inflammatory effects. They reduce ROS and suppress inflammaging, a chronic, low-grade inflammation that disrupts homeostasis, alters DNA methylation, and accelerates ageing (Bradley et al., 2025). A 2024 randomized controlled trial (RCT) (~800 mg/day polyphenols) reduced immune cell epigenetic age by ~1.9 years in 12 weeks and improved T cell profiles (Perlmutter et al., 2024). Large studies (PREDIMED, DIRECT PLUS) suggest that ≥ 1200 mg/day may offer optimal benefits (Bradley et al., 2025).

Taurine (*e.g.,* shellfish, dark-meat poultry, seaweed) and ergothioneine (*e.g.,* mushrooms, fermented pulse-based foods, oat bran), sulfur-containing compounds in mitochondria and immune cells, are emerging as anti-ageing nutrients. Taurine levels drop with age; supplementation extended lifespan in mice and correlated with lower frailty in humans (Singh et al., 2023). Low ergothioneine is linked to cognitive decline; a 16-week trial (10–25 mg/day) improved memory and telomere length (Zajac et al., 2025).

Other nutrients also support redox and epigenetic stability: vitamins C (*e.g.,* citrus fruits, berries and tropical fruits, vegetables) and E (*e.g.,* nuts and seeds, vegetable oils, greens) prevent oxidative damage; glutathione (*e.g.,* garlic, onions, eggs, whey protein) - backed by N-acetylcysteine (NAC), cysteine, and methionine - is the main redox buffer; selenium (*e.g.,* brazil nuts, seafood, sunflower seeds) and zinc (*e.g.,* nuts, seeds, whole grains, leafy greens) are cofactors for antioxidant enzymes; coenzyme Q10 and alpha-lipoic acid (*e.g.,* organ meats, spinach, whole grains), and carotenoids (*e.g.,* carrots, sweet potatoes, tomatoes) modulate mitochondrial and cellular oxidative stress (Pirayesh Islamian and Mehrali, 2015).

8.1.4. Metabolite co-substrates

Epigenetic enzymes rely on metabolite co-substrates like NAD^+, crucial for sirtuin-mediated histone deacetylation. NAD^+ declines with age but can be restored through diet (*e.g.,* polyphenol- and tryptophan-rich foods). Vitamin B3 (niacin) supports NAD^+ via the Preiss-Handler pathway, while nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) are more efficient precursors. A 2023 review confirmed NR (250–1000 mg/day) safely raises NAD^+ and may reduce inflammation, though effects on DNAm age remain uncertain (Freeberg et al., 2023).

Tryptophan (*e.g.,* dairy, turkey, eggs, soy products, nuts and seeds, legumes, whole grains) also contributes via the kynurenine pathway, which shifts toward pro-ageing kynurenic acid (KYNA) with age (Bakker et al., 2024). KYNA-rich chestnut honey rapidly boosts plasma KYNA. NAD^+ levels can also be enhanced by caloric restriction, fasting, and polyphenols like resveratrol, while apigenin preserves NAD^+ by inhibiting CD38 (Xie et al., 2020).

Another key metabolite, α-ketoglutarate (AKG), supports DNA and histone demethylation. Its levels decline with age, but Ca-AKG supplementation extended lifespan in mice and reduced DNAm age by ~8 years in humans (Demidenko et al., 2021).

The gut microbiome also shapes epigenetic ageing. Diets rich in fibre and polyphenols foster butyrate-producing microbes; butyrate acts as an HDAC inhibitor and reduces cellular senescence. Microbial NAD^+ pathways and polyphenol metabolism are emerging areas in ageing research (Iqbal and Nakagawa, 2024), with microbiome-based clocks linking gut profiles to gene expression (Gopu et al., 2024). Urolithin A, a metabolite from ellagitannins in foods like pomegranates and walnuts, improved muscle endurance and lowered inflammation in older adults (Liu et al., 2022).

8.2. Dietary patterns

Epidemiological and trial data consistently link high diet quality with slower epigenetic ageing. Diets rich in vegetables, fruits, whole grains, legumes, nuts and seeds, fatty fish, and extra virgin olive oil, and low in refined carbs and processed meats, are most protective (Gjermeni et al., 2025). Key methyl adaptogens include EGCG (tea), curcumin (turmeric), anthocyanins (berries), cocoa, olive oil phenolics, sulforaphane (cruciferous veg), and herbs like rosemary (Villanueva et al., 2025). Benefits are strongest when these foods are part of a balanced diet, not isolated supplements (Townsend et al., 2023).

8.2.1. Mediterranean diet (MedDiet)

In the NU-AGE study of 120 older adults from Italy and Poland, a one-year Mediterranean-style diet modestly reduced Horvath epigenetic age (~0.6 years), with a stronger effect in Polish women (~1.47 years). Benefits were greater in those with higher baseline epigenetic age and varied by sex and country, indicating individual responsiveness (Trajkovska Petkoska et al., 2025).

In the 18-month DIRECT-PLUS RCT (N = 294), the polyphenol-rich green Mediterranean (green-MED) diet significantly reduced biological ageing, outperforming the standard Mediterranean diet in lowering Hannum and Li DNA methylation ages by an average of 8.9 months on

the Li clock (Villanueva et al., 2025). Benefits were linked to higher polyphenol intake and improved 1-C metabolism, highlighting the green-MED diet's epigenetic impact.

8.2.2. Other diets

Beyond the Mediterranean diet, several other dietary patterns, such as the Nordic diet and traditional East Asian diets, show emerging potential in modulating epigenetic ageing, largely due to their high nutrient density, polyphenol content, and support for 1-C metabolism. While direct epigenetic clock data remain limited, their overall nutritional profiles suggest capacity to influence DNA methylation patterns and biological age (Chun et al., 2024; García-García et al., 2024b).

Additionally, the MIND diet (Mediterranean-DASH Intervention for Neurodegenerative Delay), which integrates elements of the Mediterranean and DASH diets, emphasizes berries, leafy greens, and extra virgin olive oil, while limiting red meat and added sugars. Early findings suggest that adherence to the MIND diet may slow cognitive ageing (Sawyer et al., 2024) and reduce inflammatory burden (Li et al., 2023), potentially via epigenetic pathways, although direct data on DNA methylation remain preliminary.

8.2.3. Diet quality scores

In the LifeLines DEEP cohort (~1600 adults), higher diet quality and lower intake of ultra-processed foods were linked to younger biological age via epigenetic clocks, independent of age, sex, and social determinants. Adequate B vitamin intake also supported epigenetic stability (Bordoni et al., 2024). Similar results were seen in the Women's Health Initiative (~4500 women) and WASEDA's study of older Japanese men, where traditional diets correlated with slower epigenetic ageing and longer telomeres, unlike Western diets (Kawamura et al., 2024a; Reynolds et al., 2024). A 2025 review (N = 123,923) found proinflammatory diets, measured by Dietary Inflammatory Index, often linked to shorter telomeres, though telomere length remains a less precise ageing marker (Castro et al., 2025).

8.2.4. Plant-based diet

In a Stanford pilot study of 21 identical twin pairs (~40 years old), one twin followed a vegan diet and the other an omnivorous diet for eight weeks. The vegan group showed ~1.5 years lower DNA methylation-based biological age across multiple clocks, while the omnivorous group did not (Dwaraka et al., 2024). Though promising, the study's small size and short duration limit conclusions. Plant-exclusive diets may benefit ageing through higher polyphenol and fiber intake and lower methionine but require attention to nutrients like B12, zinc, iodine, and omega-3s to avoid deficiencies (Ristori et al., 2025).

To conclude, nutrition shapes epigenetic ageing via key pathways (Table 4), especially 1-C metabolism, which regulates DNA methylation through nutrients like folate, B vitamins, methionine, choline, and betaine. Whole-food sources support balance, avoiding risks of excess supplementation. Caloric excess and high methionine intake can impair autophagy and increase oxidative stress, while caloric restriction, fasting, and methionine moderation activate protective mechanisms like sirtuins and mitochondrial renewal. Polyphenols, omega-3s, and isothiocyanates reduce inflammation and oxidative stress, preserving methylation and histone integrity. Antioxidants (*e.g.,* vitamins C/E), glutathione precursors (*e.g.,* NAC, selenium), and NAD^+ boosters (*e.g.,* niacin, NR/NMN) further support redox and epigenetic stability. Fiber- and polyphenol-rich diets promote butyrate-producing microbes that influence histone acetylation and cellular ageing. Mediterranean, Green-MED, and Japanese diets, rich in plants, fish, and polyphenols, are linked to slower biological ageing. A well-rounded, plant-forward diet supports epigenetic health. Research should clarify optimal dose, duration, and individual variability, while precision nutrition and multimodal strategies may enhance effects. The only RCT showing > 2-year DNAmAge reversal in 8 weeks combined diet with sleep, exercise, and

Table 4
Nutritional and dietary modulators of epigenetic ageing.

Mechanistic pathway/Dietary component	Representative compounds	Key epigenetic or molecular effects	Main food sources
One-carbon (1-C) metabolism	Folate (B9), Vitamins B2, B6, B12, Methionine, Choline, Betaine, Zinc, Magnesium, Serine, Glycine, Vitamin D, Creatine	Provide methyl groups for DNA methylation; support SAM synthesis; maintain methylation stability; prevent epigenetic drift; regulate homocysteine	Leafy greens, legumes, whole grains, eggs, fish, shellfish, poultry, mushrooms, nuts, seeds, dairy
Caloric and macronutrient regulation	Caloric restriction, Methionine & leucine moderation, Ketogenic diets, Intermittent fasting, β-hydroxybutyrate (βHB), Pentadecanoic acid (C15:0), Spermidine	Activate sirtuins and autophagy; inhibit mTOR/IGF-1 signalling; promote mitochondrial stability; rejuvenate DNA methylation; reduce inflammation	Plant-based and pescatarian diets; legumes; whole grains; fish; dairy; olive oil; mushrooms; aged cheese; wheat germ; fermented soy (natto)
Redox and inflammatory tone	Polyphenols (EGCG, curcumin, resveratrol, olive oil phenolics), Omega-3s (EPA, DHA), Sulforaphane, Taurine, Ergothioneine, Vitamins C/E, Selenium, Zinc, Glutathione precursors, CoQ10, α-lipoic acid, Carotenoids	Reduce ROS and 'inflammaging'; modulate DNA/histone methylation; enhance antioxidant defences; maintain mitochondrial homeostasis	Green tea, berries, turmeric, extra virgin olive oil, nuts, seeds, fatty fish, cruciferous vegetables, garlic, onions, eggs, mushrooms, citrus fruits, carrots, spinach
Metabolic co-substrates	NAD^+, Niacin (B3), NR, NMN, Tryptophan, α-ketoglutarate (AKG), Polyphenols (resveratrol, apigenin), Butyrate, Urolithin A	Support sirtuin activity and histone deacetylation; promote DNA demethylation; sustain mitochondrial biogenesis; inhibit senescence	Dairy, eggs, turkey, soy, nuts, whole grains, spinach, organ meats, pomegranates, walnuts, fermented foods, fibre-rich plant foods
Microbiome-derived metabolites	Butyrate, Urolithin A, Indoles	HDAC inhibition; promote histone acetylation; reduce cellular senescence and inflammation; improve muscle and immune function	Fibre- and polyphenol-rich foods: legumes, whole grains, fruits (esp. pomegranate, berries), nuts, vegetables
Dietary patterns	Mediterranean (MedDiet), Green-Med, MIND, Nordic, Japanese	Synergistic effects of high polyphenol, fibre, and omega-3 intake; improved 1-C metabolism; lower inflammatory burden; slower epigenetic ageing	Vegetables, fruits, legumes, whole grains, nuts, olive oil, fatty fish, green tea, herbs, seaweed, fermented soy products
Caloric restriction mimetics and adaptogens	Spermidine, Polyphenols, Ketone bodies, NAD^+ boosters	Mimic nutrient sensing pathways of fasting; enhance autophagy; protect DNA methylation and telomere length	Wheat germ, aged cheese, soy, mushrooms, tea, red wine, olive oil
Pro- vs. anti-ageing dietary balance	High sugar, ultra-processed foods (negative); high-fibre, whole-food diets (protective)	High sugar and refined carbs accelerate epigenetic ageing via IGF-1 and oxidative stress; whole-food diets slow biological ageing	Refined sweets, sugary drinks, processed meat (negative) vs. whole grains, fruits, vegetables, nuts, legumes (positive)

stress reduction (Fitzgerald et al., 2021). Future work must integrate other epigenetic marks and biomarkers (*e.g.,* CRP, IL-6) and explore novel tools like AI-based biological age estimation.

Boxmary Box 6: Nutrition

Foods and their components: To conclude, nutrition shapes epigenetic ageing via key pathways, especially 1-C metabolism, which regulates DNA methylation through nutrients like folate, B vitamins, methionine, choline, and betaine. Whole-food sources support balance, avoiding risks of excess supplementation. Caloric excess and high methionine intake can impair autophagy and increase oxidative stress, while caloric restriction, fasting, and methionine moderation activate protective mechanisms like sirtuins and mitochondrial renewal. Polyphenols, omega-3s, and isothiocyanates reduce inflammation and oxidative stress, preserving methylation and histone integrity. Antioxidants (e.g., vitamins C/E), glutathione precursors (e.g., NAC, selenium), and NAD^+ boosters (e.g., niacin, NR/NMN) further support redox and epigenetic stability. Fiber- and polyphenol-rich diets promote butyrate-producing microbes that influence histone acetylation and cellular ageing.

Diets: Mediterranean, Green-MED, and Japanese diets, rich in plants, fish, and polyphenols, are linked to slower biological ageing. A well-rounded, plant-forward diet supports epigenetic health.

Research directions: Research should clarify optimal dose, duration, and individual variability, while precision nutrition and multimodal strategies may enhance effects. The only RCT showing > 2-year DNAmAge reversal in 8 weeks combined diet with sleep, exercise, and stress reduction (Fitzgerald et al., 2021). Future work must integrate other epigenetic marks and biomarkers (e.g., CRP, IL-6) and explore novel tools like AI-based biological age estimation.

9. Computational modelling of biological age

Constructing an accurate ageing clock requires comprehensive and relevant data that capture molecular or physiological features changing predictably with age. The predictive power of ageing clock models improves with the identification of robust biomarkers of biological age. Such biomarkers can (individually or in combination) reliably estimate an organism's functional and physiological state relative to its chronological age. Recent research has focused on identifying ageing biomarkers across high-dimensional omics datasets, including genomics, epigenomics, glycomics, transcriptomics, proteomics, and metabolomics, alongside physiological and clinical parameters (Rutledge et al., 2022).

9.1. Computational methods

A wide range of machine learning and mechanistic computational models have demonstrated effectiveness in both biomarker discovery and biological age prediction (Meng et al., 2024). From a methodological view, identifying and validating key biomarkers is often treated as a feature selection problem, while the prediction of biological age typically involves regression or classification techniques. In contrast, mechanistic models aim to test biological hypotheses directly, sometimes using digital twins that simulate ageing processes at the individual level (X. Li et al., 2025).

AI and machine learning approaches have been instrumental in integrating diverse omics and clinical data to deepen our understanding of ageing. A key application is the prediction of biological age using data such as DNA methylation patterns, leukocyte telomere length, and various physiological and biochemical indicators. These models often outperform chronological age in reflecting an individual's functional state. Moreover, they facilitate the discovery of molecular features, such as specific methylation sites, telomere attrition patterns, or metabolic and inflammatory signals. These are associated with biological ageing and related diseases (Meng et al., 2024). Beyond prediction, such tools support risk stratification by identifying individuals who are biologically younger or older than their chronological age, enabling earlier interventions and advancing personalized approaches to healthy ageing.

9.2. Predicting biological age with artificial intelligence and machine learning approaches

A range of advanced AI and machine learning models have demonstrated exceptional promise in predicting biological age, leveraging diverse omics and clinical data. Machine learning and AI approaches used in predicting biological age have been extensively reviewed elsewhere (Meng et al., 2024; Ni et al., 2023), focusing on a reconstruction of qualitative model and quantitative model of biological age from a multidimensional and systematic perspective using different computational approaches. The employed models range from classical linear regression to deep neural networks and ensemble methods based on random forests and boosted trees. Furthermore, in recent years a large emphasis has been made on explainable AI (xAI) methods (Kalyakulina et al., 2024).

Linear models such as ordinary least squares regression, Lasso, Ridge, and Elastic Net are widely used for quantitative age prediction, offering a favourable trade-off between interpretability and performance (Rutledge et al., 2022). Elastic Net is a standard for DNA methylation-based clocks like Horvath's clock, known for its accuracy and interpretability (Gopu et al., 2024; Tomo and Nakaki, 2024). Despite its continued relevance, newer deep learning models are beginning to outperform Elastic Net in specific contexts. Classical regression models have also been extended into more flexible, nonparametric approaches that incorporate uncertainty estimation, such as Gaussian processes (Zhang and Yeung, 2010). These probabilistic models may further improve the reliability and confidence of biological age predictions.

On the other hand, Support Vector Machines (SVMs) are effective for handling high-dimensional omics data and capturing nonlinear associations, whereas Support Vector Regression (SVR), a variant tailored for continuous outcomes, is a strong performer in age prediction and often serves as a benchmark for accuracy in high-dimensional settings (Meng et al., 2024). Furthermore, these models can be combined with other models in ensemble learning approaches (Couvy-Duchesne et al., 2020).

Non-linear machine learning methods have been used to capture the complex, multidimensional nature of ageing processes. However, the validity of their outputs remains untested (Mei et al., 2025). *PathwayAge* integrates biological pathway information alongside epigenetic data within a two-stage regression framework, enabling the examination of disease associations and offering improved interpretability (P. Li et al., 2025).

Ensemble learning approaches include random forests that are highly interpretable and robust, making them effective for identifying key biomarkers and predicting biological age. Bagged trees and boosted trees are top performers in both qualitative and quantitative biological age prediction, offering strong accuracy and the ability to handle nonlinear relationships between features and age (Malli et al., 2016).

Deep learning architectures, including Convolutional Neural Networks (CNNs) and Recurrent Neural Networks (RNNs), have shown promise in extracting complex patterns from omics data, particularly for image-based or sequential data (Wilczok, 2025). For instance, *DeepAge* employs a temporal convolutional network that models methylation data as sequential input, yet it currently lacks longitudinal validation and biological verification (Mei et al., 2025). Deep Neural Networks (DNNs) consistently outperform traditional statistical methods, achieving high predictive accuracy for biological age across various data types, such as blood markers, DNA methylation, and imaging.

9.3. Predicting biological age with explainable artificial intelligence methods

While deep learning and other complex AI models offer high

predictive accuracy, they often operate as "black boxes," making it difficult to interpret how predictions are made and to translate findings into actionable biological or clinical insights (P. Li et al., 2025; Pyrkov et al., 2018). To address this limitation, there has been a strong shift toward explainable and interpretable AI (xAI). xAI approaches aim to uncover the biological mechanisms behind ageing predictions by developing interpretable models or applying post-hoc explanation techniques (Bernard et al., 2023; Wilczok, 2025). These frameworks help identify key predictive features and biological pathways, bridging the traditional trade-off between accuracy and interpretability.

SHAP-based post-hoc explanations have been employed in *AltumAge* for elucidating feature importances, though the biological interpretability of its findings remains unclear (Mei et al., 2025).ENABL Age framework combines high-performance machine learning with explainable AI to deliver both accurate biological age estimates and individualized explanations, enhancing clinical utility (Qiu et al., 2023). Another example is XAI-AGE, an interpretable deep neural network that integrates biological pathway information for DNA methylation-based age prediction, offering both precision and biological insight (Wilczok, 2025).

Another example is XAI-AGE, a biologically informed and interpretable deep neural network for DNA methylation-based age prediction that integrates hierarchical biological pathway information across multiple tissue types. It enables multimodal input extensions at the gene level and allows direct comparison and inference of relationships between abstract data layers, offering both precision and biological insight; however, its training still relies on chronological age (Prosz et al., 2024; Wilczok, 2025).

Li et al. (2025) recently developed another deep learning framework paired with explainable AI (Z.-P. Li et al., 2025). The deep learning framework was used to construct molecular ageing clocks at cell-type resolution using both mRNA expression and DNA methylation data from immune cells. The architecture used was a multi-view graph-level representation learning (MGRL) framework combining deep graph convolutional networks with multilayer perceptrons. Meaningful biological insights were extracted using adapted PGExplainer algorithm, which enabled global interpretability of graph neural network (GNN) predictions (Luo et al., 2020). Although the proposed model performed only marginally better than classical alternatives, its interpretability provided valuable mechanistic insights into cell-type-specific ageing patterns (Z.-P. Li et al., 2025).

Together, these studies reflect a broader trend toward biologically informed and interpretable deep learning frameworks in epigenetic clock research, seeking to retain predictive performance while enhancing interpretability.

9.4. Towards causality in biological ageing models

AI approaches have made impressive strides in balancing predictive accuracy with interpretability, however, not providing direct mechanistic or causal explanations. Moving beyond interpretability to causality is critical for understanding the underlying drivers of ageing and for designing effective interventions. This transition calls for integrating causality-aware methods into biological ageing models, combining statistical inference with mechanistic knowledge to disentangle cause-effect relationships. Advances in causal inference in (multimodal) ageing clocks have paved the way for mechanistically informed models of biological ageing.

Beyond model interpretability, some research has focused on uncovering causal relationships between biological ageing and ageing-related phenotypes. For instance, Hao et al. (2024) investigated potential causal links between biological ageing, Alzheimer's dementia, and cognitive function using Mendelian randomization (MR) methods (Hao et al., 2024). MR approaches are particularly valuable in this context, as they can mitigate confounding biases that often limit traditional epidemiological studies. In a complementary effort, Ying et al. (2024) employed epigenome-wide Mendelian randomization to infer causal relationships between DNA methylation modifications and ageing-associated traits, both detrimental as well as adaptive. This study identified a set of causal CpG sites, laying the groundwork for the development of causality-informed epigenetic clocks that go beyond correlational modelling (Ying et al., 2024).

9.5. Mechanistic models of ageing

While causality-enriched ageing clocks provide valuable insights into potential drivers and protective factors of biological ageing through statistical and genetic inference, they often remain agnostic to the underlying biological pathways and dynamic interactions. Bridging this gap requires mechanistic models that explicitly represent molecular and cellular processes responsible for ageing phenotypes. Mechanistic models enable hypothesis testing and simulation of interventions by capturing causal mechanisms at scales ranging from metabolic networks to cellular signalling and clock regulation. Thus, integrating causality-informed biomarkers from multimodal ageing clocks with mechanistic frameworks offers a promising avenue towards a more complete understanding of ageing biology. Even though comprehensive mechanistic models of ageing are scarce, smaller models focused on specific aspects of ageing have been established. For example, ageing clocks based on accumulating stochastic variation have been proposed (Meyer and Schumacher, 2024). Another study established physiological links between the circadian clock and ageing clock (Liu and Chang, 2017). The most comprehensive attempts to describe the process of ageing in a systematic way to date are probably genome-scale metabolic models.

Genome-scale metabolic models (GEMs) have been applied to study the metabolic hallmarks of healthy ageing and extreme longevity, offering valuable insights into the metabolic shifts that occur during ageing (Li et al., 2022). In model organisms such as *Caenorhabditis elegans* and mice, GEMs integrated with multi-omics data-including transcriptomics and metabolomics-have revealed significant age-related metabolic changes. For instance, in *C. elegans*, metabolic flux analysis uncovered imbalances in the tricarboxylic acid (TCA) cycle and amino acid metabolism during ageing, with enhanced prediction accuracy achieved by incorporating metabolomics data into the models (Hastings et al., 2019). In mice, integrated host-microbiome metabolic models have demonstrated that ageing reduces microbiome metabolic activity and alters host-microbiome metabolic interactions, contributing to systemic metabolic decline in key tissues such as the colon, liver, and brain (Best et al., 2025). Furthermore, GEMs have been instrumental in identifying metabolic pathways that become dysregulated in age-related diseases by analysing metabolic modules that are either induced or repressed with ageing. The Metabolic Transformation Algorithm (MTA) has also been applied to GEMs to predict potential metabolic drug targets for age-related diseases by identifying interventions capable of reversing disease-associated metabolic states (Yizhak et al., 2013). These approaches collectively advance our understanding of the metabolic underpinnings of ageing and offer promising avenues for therapeutic development.

Multi-omic approaches have also been employed to deepen the understanding of the molecular architecture underlying ageing-related traits. Mavromatis et al. (2023) conducted a comparative multi-omic analysis of epigenetic age acceleration and a multivariate longevity-related phenotype, integrating data from genome-wide association studies (GWAS) of four epigenetic clocks with transcriptome-wide association studies (TWAS). This integrative framework enabled the identification of novel gene associations and the characterization of transcriptomic effects linked to previously established genetic loci. By jointly investigating biomolecular associations across genomic and transcriptomic layers, the study provided new insights into the shared and distinct molecular pathways influencing both accelerated epigenetic ageing and longevity. Overall, these multi-omic analyses facilitated biological comparisons between ageing

phenotypes, offering a systems-level view of how genetic and transcriptomic factors interact to shape ageing trajectories (Mavromatis et al., 2023).

9.6. Practical advances and applications

9.6.1. Limitations

Despite significant progress, several challenges remain in the development, validation and application of biological ageing clocks.

A major obstacle is the heterogeneity of omics data, which vary in scale, noise, and patterns of missingness, complicating both integration across data modalities and generalization of models. Additionally, many ageing clocks are developed using limited or demographically homogeneous cohorts, raising concerns about their robustness and applicability to broader, more diverse populations. For example, most genome-wide association data originate from European ancestry populations, such as UK Biobank participants, and reflect environmental and medical conditions from past decades, thereby reducing trans-ancestral and temporal relevance (Mavromatis et al., 2023). Validation remains a persistent challenge, with few longitudinal or multi-ethnic cohorts available for testing models (Z.-P. Li et al., 2025; Mavromatis et al., 2023).

Many machine learning approaches rely on transformations that impose a linear structure onto data to improve prediction accuracy; however, biological ageing is inherently nonlinear. Consequently, linear models such as Elastic Net regression may fail to capture ageing trajectories, as DNA methylation changes do not occur linearly over time. Moreover, such models do not imply causation and may obscure key molecular features that distinguish healthy ageing from disease-related deviations within the same chronological age group (Mei et al., 2025).

While advanced models such as deep learning and ensemble methods achieve high predictive accuracy, they often lack interpretability, making it difficult to extract meaningful biological insights from their outputs (Meng et al., 2024; Rutledge et al., 2022).

Finally, the lack of standardized protocols and open-access benchmarks for data preprocessing, feature selection, and model validation hampers reproducibility and comparability across studies, limiting the broader utility and trustworthiness of current approaches (Rutledge et al., 2022). To address this gap, Kriukov *et al.* (2025) introduced *ComputAgeBench*, the first systematic benchmarking framework for epigenetic ageing clocks. It integrates multiple evaluation tasks to produce cumulative performance scores across 66 public DNA methylation datasets (Kriukov et al., 2024).

The interpretability of model outputs can also be compromised when features have weights that do not agree with their univariate correlations with the response variable, potentially distorting the biological meaning of the predicted values (Mei et al., 2025).

Moreover, current biological clocks do not yet fully exploit the wide array of available molecular modalities or functional data, and most are based on cross-sectional rather than longitudinal data. The extent to which these clocks capture correlational versus causal aspects of ageing remains poorly understood. Elucidating causal relationships between ageing-related factors, health outcomes, and underlying molecular mechanisms will be essential for refining biological clocks and guiding the development of effective interventions (Rutledge et al., 2022).

Additionally, the field still lacks systematic studies on biological noise, though some evidence suggests that intrinsic variability in DNA methylation may encode meaningful biological information (Mei et al., 2025).

9.6.2. Emerging trends

To facilitate research in this area, Ying et al. (2023) introduced ClockBase, a computational platform that integrates multiple ageing clocks, provides comparative insights into their relationships, and offers an accessible user-friendly interface for broader application in the ageing research community (Ying et al., 2023). Emerging trends in the field of biological ageing clocks reflect a shift toward more holistic, adaptable, and predictive approaches. Multi-omics integration is gaining traction, aiming to combine data from genomics, epigenomics, transcriptomics, proteomics, and metabolomics to capture a more comprehensive view of the ageing process (Rutledge et al., 2022). At the same time, some studies are emphasizing the importance of analysing the variability of biomarkers with ageing and disease, suggesting that inherent noise within DNA methylation data may itself carry biological meaning and could be leveraged to build predictive models (Mei et al., 2025). Transfer learning and domain adaptation are increasingly employed to enhance model performance, particularly when working with small or heterogeneous ageing cohorts by leveraging insights from related datasets or tasks. Additionally, there is a growing move toward longitudinal modelling, which enables the analysis of ageing trajectories over time and supports the prediction of future health outcomes, moving beyond traditional cross-sectional analyses (Rutledge et al., 2022).

In summary, AI and machine learning are revolutionizing the study of biological ageing by enabling the integration and analysis of complex omics and clinical data. Methods such as ensemble models, support vector machines, neural networks, and regularized regression have shown strong performance in predicting biological age and identifying ageing biomarkers. Despite this progress, challenges in data integration, interpretability, and generalizability remain central concerns. Emerging directions, including multi-omics integration, explainable AI, and longitudinal modelling, hold great promise for advancing the field and translating computational findings into actionable strategies for promoting healthy ageing (Rutledge et al., 2022).

10. Conclusion

Numerous studies have analysed different aspects of biological age and developed clocks and models to assess biological age and measure the molecular changes due to biological ageing. Not only are there several generations of epigenetic clocks used to estimate biological age, but proteome-based clocks were developed, and metabolome- and microbiome-based clocks are being developed as well. Genomic studies have uncovered several genetic mechanisms that promote longevity, with a focus on protective mechanisms such as protective genetic variants and effective DNA repair systems. Epigenomic changes that influence biological age are modified by diet and exercise and influenced by early life events. Age-related changes in blood proteome were identified, revealing non-linear and organ-specific alterations. Metabolomic profiles in blood plasma have identified age-related shifts in lipid metabolism and redox balance and demonstrated their application as biomarkers for ageing processes and health outcomes. Microbiomics has shown that the uniqueness and diversity of the gut microbiome reflect biological age and that this can also be measured by microbiome derived metabolites in plasma. In addition, multi-omics approaches have uncovered potential biomarkers that not only reflect the ageing processes but can also serve as targets for personalised interventions.

The latest discoveries in the field of biological ageing are already being translated into actionable prevention strategies. Epigenetic clocks such as GrimAge and FitAge have shown that epigenetic ageing is slowed down by lifestyle interventions such as exercise. High levels of physical fitness and physical activity improve all aspects of biological ageing. Long-term dietary habits may be encoded in the epigenome and numerous studies have confirmed the mechanistic link between nutrition and the epigenome. A plant-based diet has been shown to slow down epigenetic ageing. In addition, there is increasing evidence of close interactions between metabolome, microbiome, epigenome, fitness and nutrition that can influence the direction and speed of biological ageing. Computational modelling, especially machine learning and explainable AI, can integrate diverse data on biological ageing, predict biological age and stratify individuals according to ageing risks. These tools will enable researchers and clinicians to develop tailored healthy ageing programmes that combine unique genetic profiles,

lifestyle factors and environmental exposures.

There are several limitations in selecting reliable biomarkers of ageing. First, there is a lack of consistently identified biomarkers, low methodological standardisation, and limited numbers of cohorts in ageing studies. Currently, ageing appears to be a non-linear process that does not progress at the same rate across all biological functions and organs. Comparisons of different clocks and omics data have shown poor correlation, suggesting that each clock or omics may represent a distinct ageing process. There is limited translation of DNA methylation and other biomarkers into clinical practice. Furthermore, the definition of biological ageing is not yet clearly established within the community. Therefore, relying on only one type of data is unlikely to provide precise, specific, and reliable biomarkers. Ageing is a systems-level biological process, and only systems-level approaches are likely to lead to the development of reliable and interpretable predictions of biological age. Comparison of different omics data has also shown poor correlation between different molecular domains, indicating that each domain may reflect a different ageing process or organ. Moreover, it is clear that individuals and their organs age differently and at different rates.

However, to advance the development of tailored ageing programmes, we need more comprehensive longitudinal clinical studies that include multiple factors (environment, lifestyle, genome) that influence biological ageing in different populations. Studies on prevention strategies should include more detailed information using tools to monitor diet, exercise and sleep habits and include individual variability. We need to go beyond epigenetic clocks and include novel biomarkers and AI-based tools. Only then can we begin to develop personalised prevention strategies to slow down biological ageing and predict individual ageing trajectories.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

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