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APPLICATION NOTE

NAGI™ Biological Age

Multidimensional Phenotypic Profiling of Healthy Aging Interventions

Using the powerful preclinical model *C. elegans* for research on healthy aging.

Core interventions: metformin, high-glucose diet, their combination, NAM & sodium butyrate,

Scientific question: How do different healthy-aging interventions influence biological aging trajectories, and which physiological systems contribute to their observed effects?

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Advancing the evaluation of healthy aging interventions

Aging is a multifactorial biological process involving interconnected mechanisms, including genomic instability, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, and dysbiosis [1]. The expanded hallmarks of aging framework highlights what a multifaceted process aging is and the importance of assessing interventions across complementary biological levels. Accordingly, healthy aging research is increasingly moving beyond lifespan and individual molecular markers toward multidimensional assessments integrating physiological function, multi-omics, and artificial intelligence (AI) [2].

Despite these advances, translating promising interventions into meaningful human health benefits remains challenging. Long-term clinical outcomes require extensive follow-up, while biological age biomarkers still require further standardization and validation against clinically relevant endpoints [3]. These challenges highlight the importance of scalable preclinical models that can characterize effects associated with interventions, identify distinct physiological responses, and prioritize candidates for subsequent investigation. In parallel, evolving regulatory approaches, including the development and evaluation of New Approach Methodologies (NAMs), emphasize the importance of reliable, biologically relevant non-clinical evidence [4].

In this context, *C. elegans* provides a well-established model for aging research, combining a short lifespan, conserved longevity-associated pathways, and suitability for scalable phenotypic screening [5]. Building on these advantages, **NAGI™ Biological Age integrates automated longitudinal phenotyping with AI-powered analysis to quantify biological aging at the whole-organism, physiological system, and organ/function levels**.

In this Application Note, we illustrate how this multidimensional framework can characterize responses to selected compounds and metabolic interventions, providing complementary phenotypic insights for early-stage healthy aging research.

Explore the Technical Note on NAGI™ Biological Age

NAGI™ Biological Age at a glance

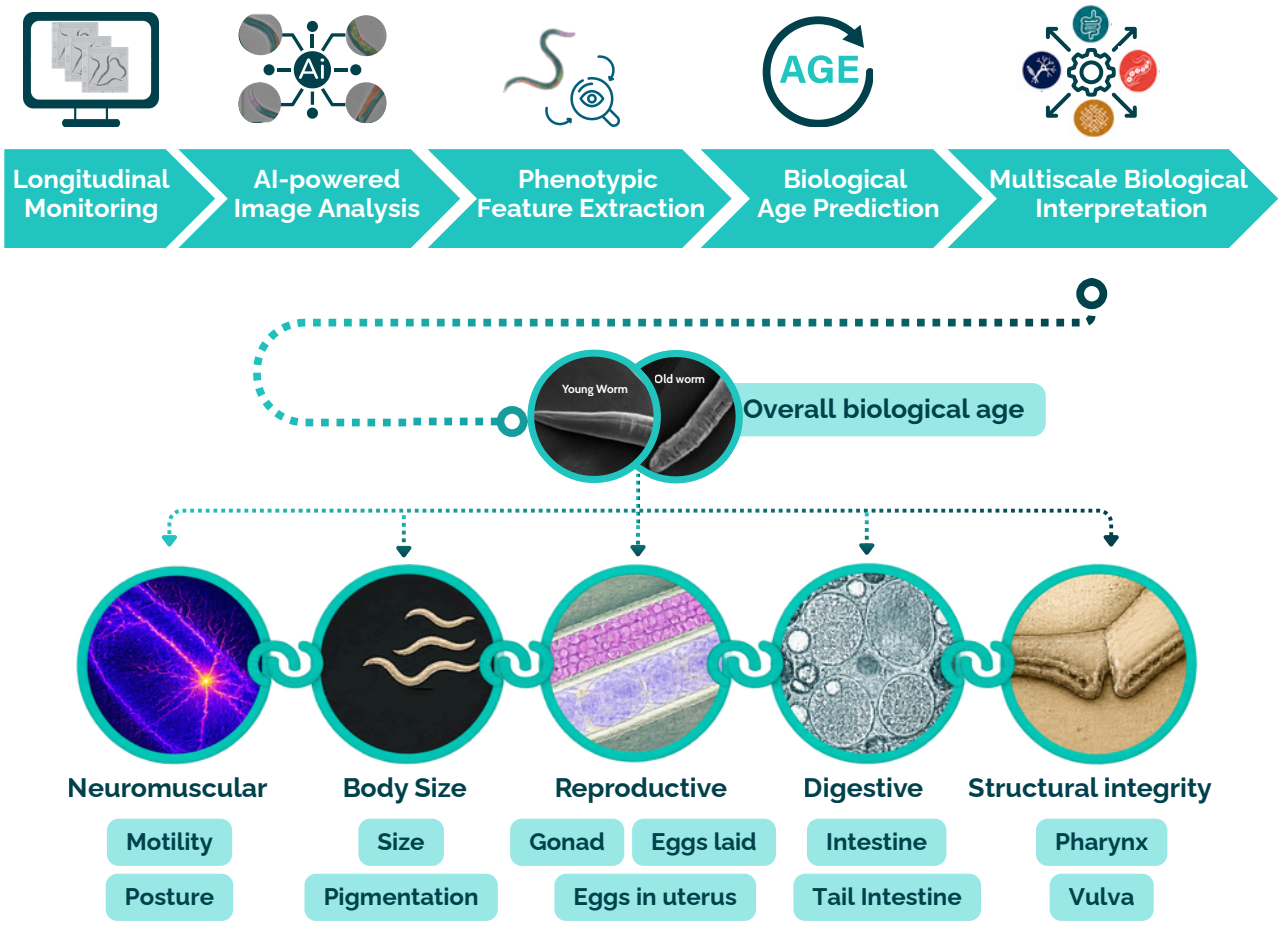


Figure 1. NAGI™ Biological Age workflow and multidimensional phenotypic assessment. Automated longitudinal imaging of *C. elegans* enables the extraction of more than 60 behavioral, morphological, and anatomical features. AI-driven analysis generates an overall biological age estimate relative to a selected control and resolves its physiological contributions across systems, organs, and functions.

NAGI™ Biological Age combines automated longitudinal phenotyping of *C. elegans* with AI to quantify intervention-associated changes in biological aging. Using the SydLab™ One platform, repeated non-invasive brightfield imaging captures behavioral, morphological, and anatomical changes throughout adulthood. More than 60 phenotypic features are integrated into an AI-derived biological age estimate relative to a selected control trajectory.

Beyond a single aging score, an interpretable AI framework resolves biological age into five major physiological domains and their underlying organ- and function-level readouts. This multiscale approach enables researchers to characterize how interventions influence overall aging trajectories and identify the physiological features contributing to the observed responses.

Given the diversity of biological processes involved in aging, interventions targeting different pathways may produce distinct physiological outcomes. To investigate these differences, we applied NAGI™ Biological Age to selected compounds and metabolism-related interventions, including **nicotinamide (NAM), sodium butyrate, a high-glucose diet, metformin, and their combination,** assessing their effects on longitudinal biological aging and the contribution of individual physiological systems.

Different interventions reshape biological age differently, with distinct magnitudes, timings, and physiological signatures.

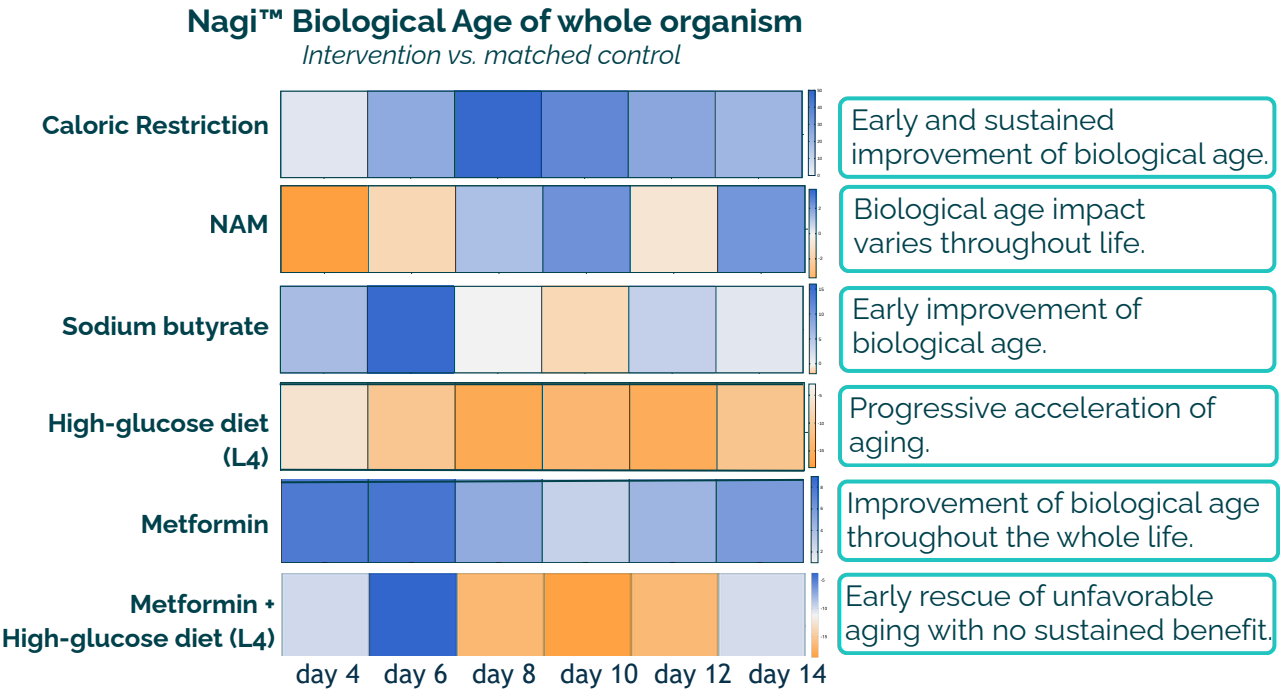


Figure 2. Nagi™ Biological Age evolution in response to different interventions, including caloric restriction as a positive control for longevity. Biological age (days) was normalized to the corresponding negative control, and the biological age magnitude was compared to the negative control throughout whole life.

NAM

Nicotinamide (NAM), a form of vitamin B3 involved in NAD^+ metabolism, has been investigated for its role in regulating longevity-associated pathways. Studies in *C. elegans* have reported lifespan extension at selected concentrations [6]. **Multidimensional biological age measurements provide an opportunity to investigate whether NAM-associated phenotypic responses are consistent across physiological systems or sustained throughout adulthood.**

NAM reveals time-dependent changes in biological age, mainly driven by eggs laid

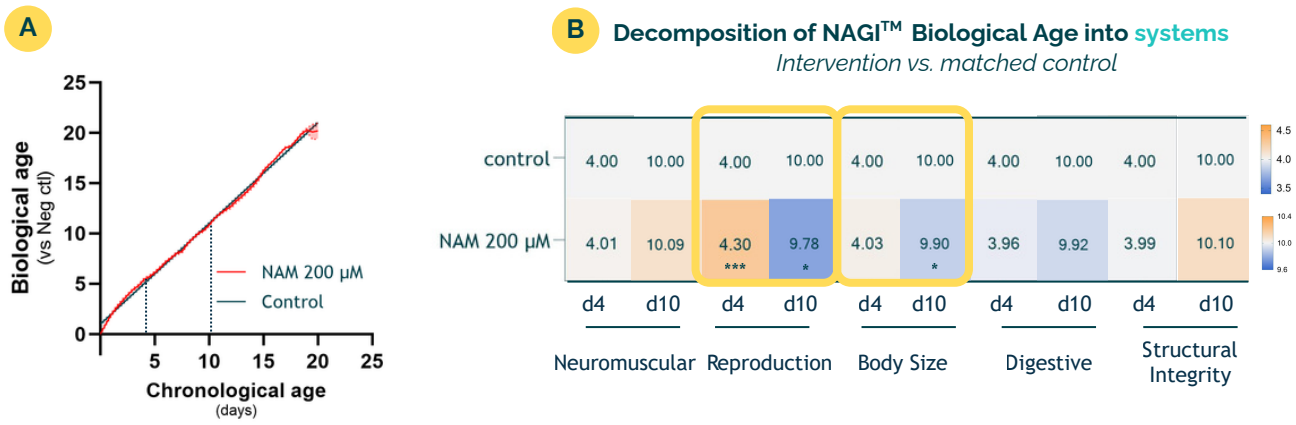
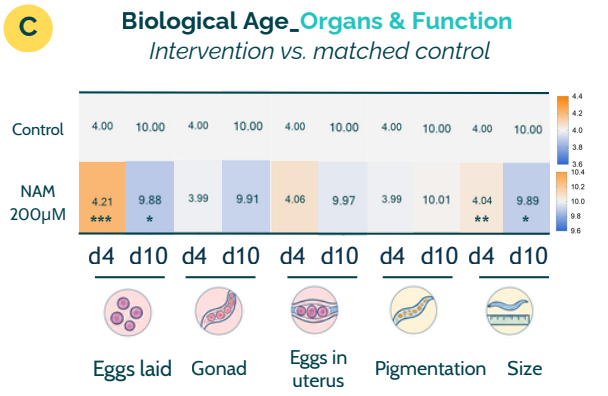


Figure 3. Nagi™ Biological Age evolution in response to NAM. Biological age (days) was normalized to the corresponding negative control and plotted as a function of chronological age (days) (A). The biological age magnitude of the systems was compared to the negative control at early (day 4) and late (day 10) time points and illustrated with a heatmap (B). The biological age magnitude of the organs affected, corresponding to the reproductive system and body size, was also compared to the negative control at days 4 & 10 (C). Values were obtained by comparing the negative control with the conditions using a two-sided Mann-Whitney U test (*p < 0.05, **p < 0.001, ***p < 0.0001, ****p < 0.00001).



NAM treatment revealed a time-dependent biological age response. During early adulthood, reduced egg-laying contributed to an older biological age profile (B). At later time points, this trend reversed, with egg-laying activity and body size contributing to a younger biological age profile (C). **These findings highlight how reproductive dynamics can differentially influence biological age predictions throughout adulthood, emphasizing the importance of longitudinal phenotypic assessment.**

Sodium Butyrate

Sodium butyrate, a microbiota-associated short-chain fatty acid and histone deacetylase inhibitor, has been investigated for its effects on longevity and intestinal proteostasis. Studies in *C. elegans* have reported lifespan extension and protection against bacteria-associated intestinal protein aggregation under specific experimental conditions [7, 8]. **In our study, sodium butyrate contributed to a younger biological age profile early in life through favorable reproductive and body-size phenotypes.**

Early biological improvement driven by digestive and reproductive changes

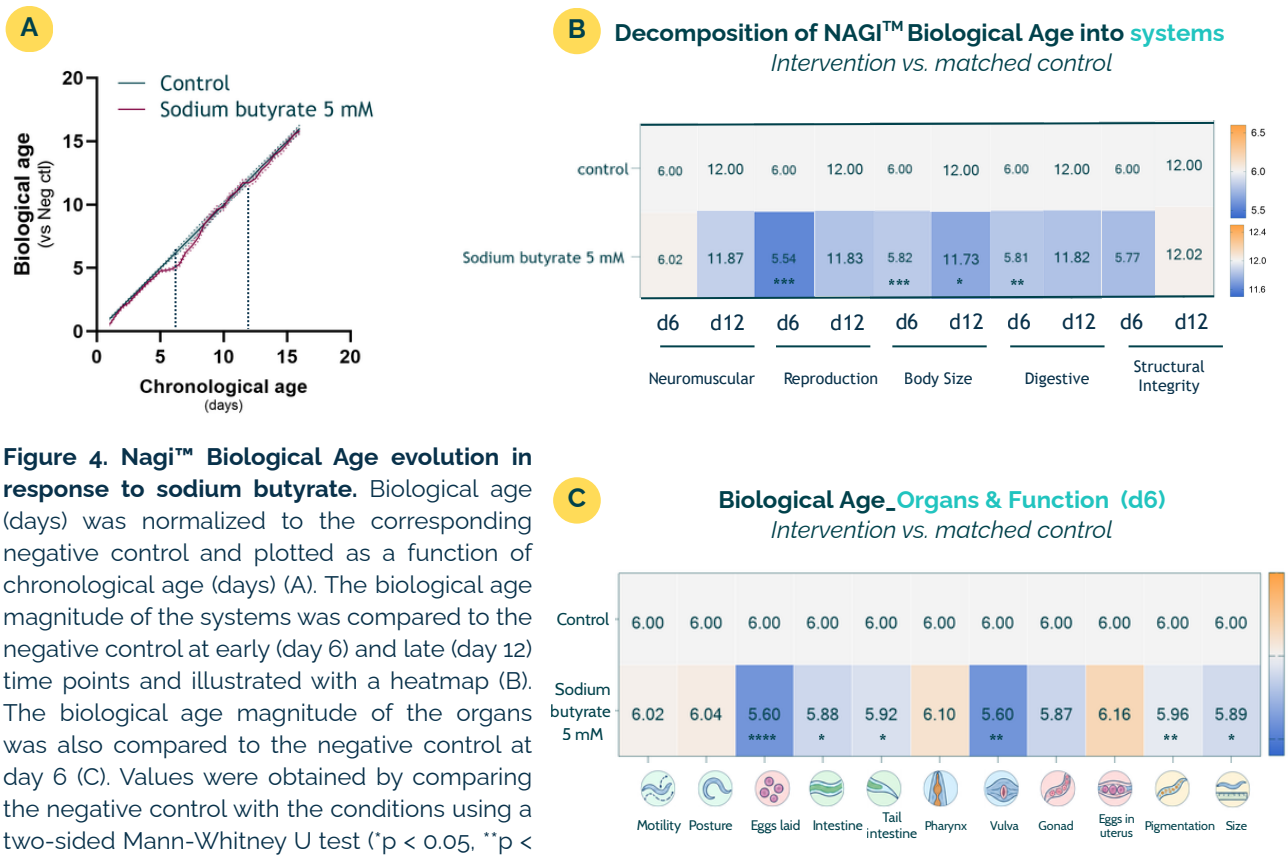


Figure 4. Nagi™ Biological Age evolution in response to sodium butyrate. Biological age (days) was normalized to the corresponding negative control and plotted as a function of chronological age (days) (A). The biological age magnitude of the systems was compared to the negative control at early (day 6) and late (day 12) time points and illustrated with a heatmap (B). The biological age magnitude of the organs was also compared to the negative control at day 6 (C). Values were obtained by comparing the negative control with the conditions using a two-sided Mann-Whitney U test (*p < 0.05, **p < 0.001, ***p < 0.0001, ****p < 0.00001).

Sodium butyrate treatment revealed a transient reduction in biological age during early adulthood, with favorable contributions from digestive, reproductive, and body-size systems (B). At the organ and function level, these responses were primarily associated with intestinal morphology, eggs laid, vulval morphology, and pigmentation. **However, these favorable effects were not sustained at later time points, highlighting the importance of longitudinal assessment in distinguishing transient from persistent physiological aging responses.**

High glucose diet

High-glucose diets provide a model for investigating the effects of nutrient excess on organismal aging. In *C. elegans*, glucose enrichment has been associated with reduced lifespan through alterations in nutrient-sensing pathways, including DAF-16/FOXO signaling [9]. Recent studies have also reported accelerated reproductive aging, with insulin-like signaling in intestinal and muscle tissues contributing to these effects [10]. **Importantly, responses can vary depending on the timing of glucose exposure [11], highlighting the relevance of longitudinal, multidimensional phenotyping for characterizing dietary challenges.**

High glucose diet

Timing of high-glucose diet exposure shapes biological age

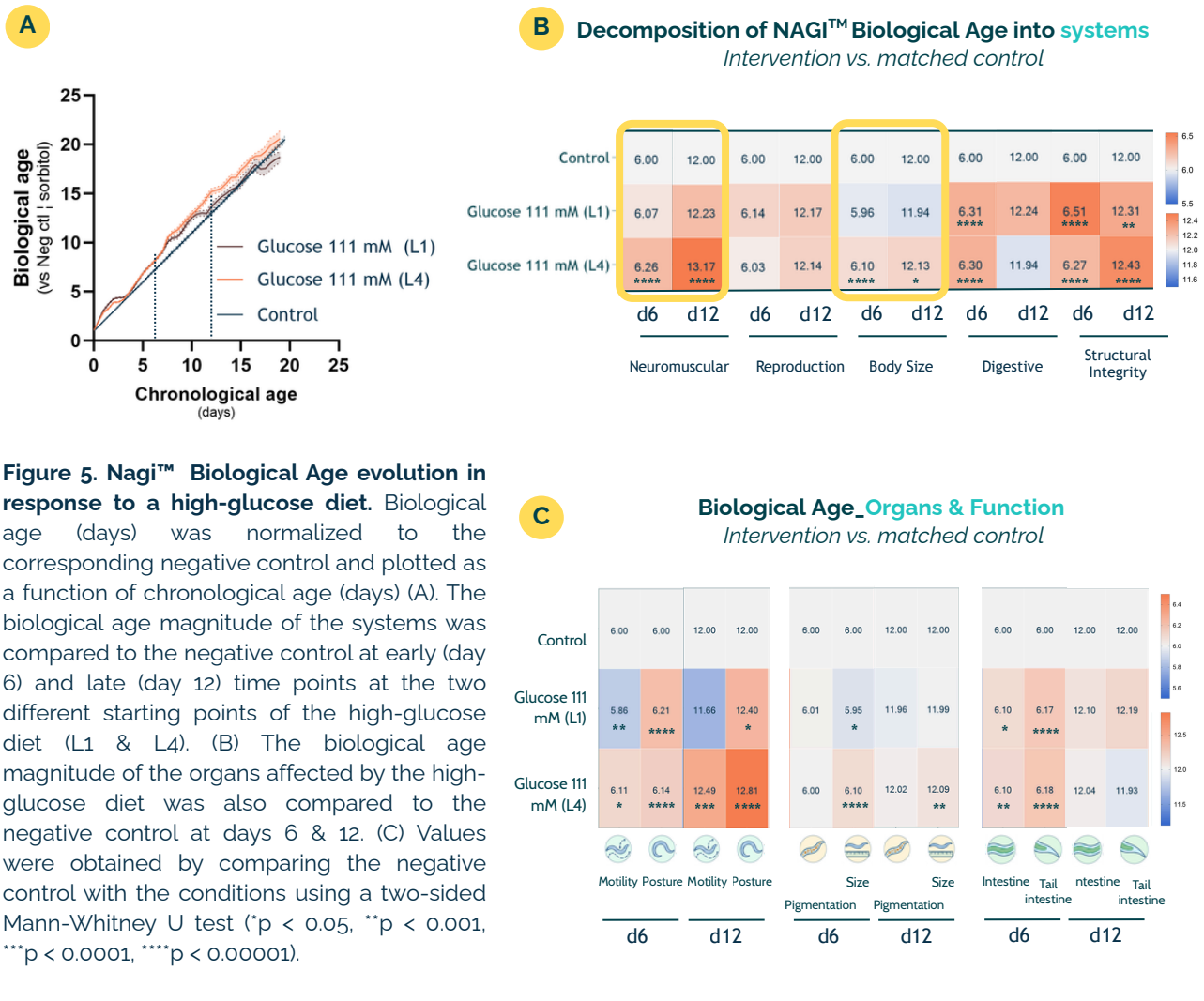


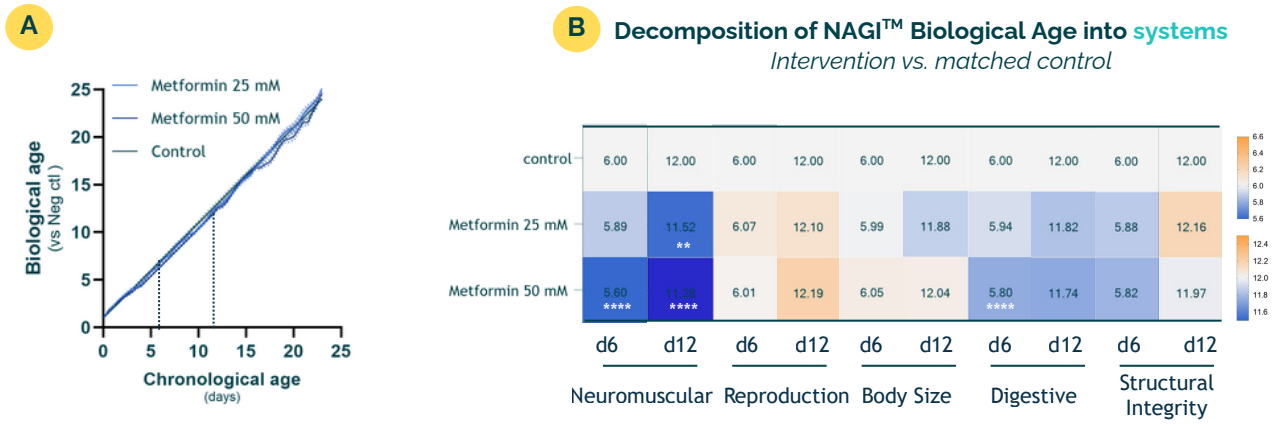
Figure 5. Nagi™ Biological Age evolution in response to a high-glucose diet. Biological age (days) was normalized to the corresponding negative control and plotted as a function of chronological age (days) (A). The biological age magnitude of the systems was compared to the negative control at early (day 6) and late (day 12) time points at the two different starting points of the high-glucose diet (L1 & L4). (B) The biological age magnitude of the organs affected by the high-glucose diet was also compared to the negative control at days 6 & 12. (C) Values were obtained by comparing the negative control with the conditions using a two-sided Mann-Whitney U test (*p < 0.05, **p < 0.001, ***p < 0.0001, ****p < 0.00001).

High-glucose diets revealed distinct physiological aging responses depending on exposure time (L1 or L4 developmental stage) (B). Worms exposed from L1 showed improved motility during early adulthood, partially counterbalancing unfavorable posture-related changes, whereas worms exposed from L4 exhibited impaired motility, contributing to an overall older neuromuscular age profile (C). The size of L4-treated worms was negatively affected throughout life, while L1-treated worms showed a modest early improvement. Both conditions exhibited unfavorable digestive aging responses during early adulthood, alongside persistent changes in structural integrity throughout adulthood. **These findings highlight how the timing of dietary exposure influences the magnitude and physiological distribution of aging-related phenotypes.**

Metformin

Metformin is a widely studied metabolic modulator associated with longevity research and an anti-diabetic medication. In *C. elegans*, previous studies have reported dose-dependent effects on lifespan, locomotor performance, and reproductive activity, with outcomes influenced by bacterial metabolism and dietary conditions [12,13]. These observations make metformin a relevant reference intervention for investigating how biological age responses evolve with treatment concentration and across physiological systems. **Longitudinal phenotyping enables the assessment of whether changes in overall biological age are accompanied by distinct neuromuscular, reproductive, or morphological aging profiles.**

Dose-dependent reduction in biological age, with key contributions from neuromuscular and digestive systems



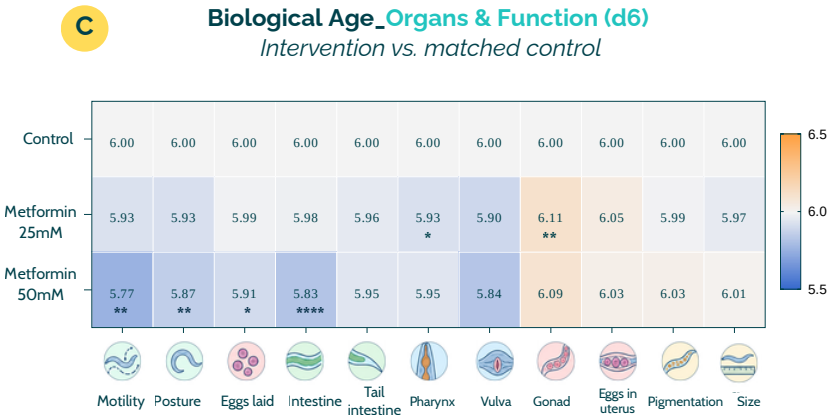


Figure 6. Nagi™ Biological Age evolution in response to metformin. Biological age (days) was normalized to the corresponding negative control and plotted as a function of chronological age (days) (A). The biological age magnitude of the systems was compared to the negative control at early (day 6) and late (day 12) time points and illustrated with a heatmap. (B) The biological age magnitude of the organs was compared to the negative control at day 6 and illustrated with a heatmap. (C) Values were obtained by comparing the negative control with the conditions using a two-sided Mann-Whitney U test (*p < 0.05, **p < 0.001, ***p < 0.0001, ****p < 0.00001).

Metformin + High-glucose diet

Metformin was tested at two doses, 25 mM & 50 mM, revealing a dose-dependent expansion in biological age. At the highest concentration (50 mM), neuromuscular and digestive systems were the main contributors to this effect (B). **While the neuromuscular response was sustained throughout adulthood, the digestive contribution was most pronounced at earlier time points, particularly until day 6. Further analysis at the organ level identified intestinal morphology as the primary contributor to the digestive system response until day 6** (C).

Metformin + High-glucose diet

Having characterized the effects of metformin and high-glucose exposure independently, we next investigated whether metformin could rescue the unfavorable aging phenotypes associated with a high-glucose diet.

Metformin as a potential rescue intervention under high-glucose diet

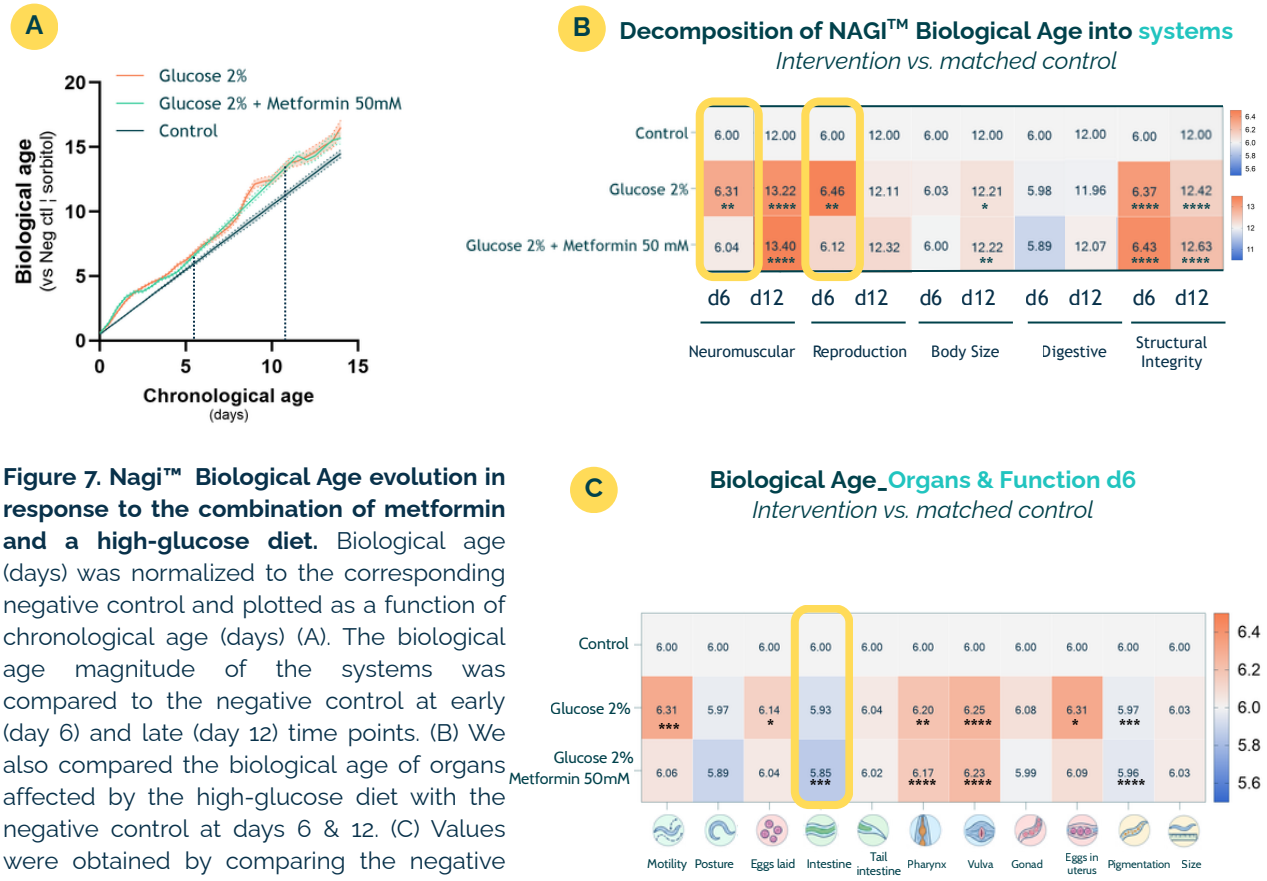


Figure 7. Nagi™ Biological Age evolution in response to the combination of metformin and a high-glucose diet. Biological age (days) was normalized to the corresponding negative control and plotted as a function of chronological age (days) (A). The biological age magnitude of the systems was compared to the negative control at early (day 6) and late (day 12) time points. (B) We also compared the biological age of organs affected by the high-glucose diet with the negative control at days 6 & 12. (C) Values were obtained by comparing the negative control with the conditions using a two-sided Mann-Whitney U test (*p < 0.05, **p < 0.001, ***p < 0.0001, ****p < 0.00001).

Metformin co-treatment revealed a partial rescue of high-glucose-associated phenotypes, with an improvement in neuromuscular response and reproduction observed at day 6. However, this effect was not sustained at day 12, indicating a transient benefit. Further analysis of individual phenotypic readouts of organs at day 6 revealed improvements in intestinal morphology (C). **These findings highlight how multidimensional biological age assessment can identify tissue-specific responses and distinguish transient from sustained effects of potential rescue interventions.**

Conclusion

The results demonstrate the ability of NAGI™ Biological Age to capture distinct, intervention-specific aging responses across multiple physiological systems and throughout adulthood. Metformin revealed dose-dependent improvements in biological age, while NAM and sodium butyrate highlighted how physiological contributions can vary over time, with both favorable and unfavorable responses depending on the stage of adulthood. High-glucose exposure further demonstrated the importance of intervention timing, revealing distinct aging phenotypes depending on the developmental stage at which treatment was initiated. Notably, metformin co-treatment partially rescued glucose-associated neuromuscular alterations during early adulthood, although this effect was not sustained at later time points.

Together, these findings emphasize that changes in overall biological age do not necessarily reflect uniform responses across physiological systems. By integrating longitudinal monitoring with organ- and function-level phenotyping, NAGI™ Biological Age enables researchers to identify dose-dependent effects, distinguish transient from sustained responses, and uncover physiological contributions that may remain hidden within a single aging score.

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