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Original Research

Social Interaction With Younger Females Extends Lifespan in Aged Male *Drosophila melanogaster* Independent of Vitamin C

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Abstract

Background: Aging is a multifactorial process shaped by environmental, social, and nutritional influences that collectively drive molecular and physiological decline. The fruit fly *Drosophila melanogaster* is a well-established model for aging research due to its short lifespan and the conservation of key regulatory pathways across species. While many interventions target single mechanisms, the combined effects of nutritional antioxidants, such as vitamin C, and social interactions on stress responses, lifespan, and functional aging remain insufficiently understood. Therefore, this study aimed to investigate the effects of social interaction and vitamin C supplementation on aging male fruit flies. **Methods:** The *D. melanogaster Oregon R(R)* strain was used, with 14-day-old males designated as aged flies and 1–3-day-old females as young flies. This sex-specific design accounted for established differences in lifespan and reproductive biology between males and females. Co-housed groups were maintained on a standard diet with or without vitamin C supplementation at concentrations of 1.25, 2.5, and 5 mM. Lifespan, endurance, and locomotor performance were assessed in aged males. In parallel, reverse transcription quantitative polymerase chain reaction (RT-qPCR) analyses were conducted to examine gene expression across functional categories, including stress response, antioxidant defense, metabolism, and neurotransmitter-related pathways. **Results:** Co-housing with young females significantly extended the lifespan of aged males, whereas the addition of vitamin C did not confer further benefit. Notably, vitamin C supplementation reduced endurance at certain concentrations, while locomotor activity remained unaffected. Gene expression analyses indicated that the observed lifespan extension was primarily associated with the co-housing condition, characterized by modulation of stress-response pathways and increased expression of *ddc*, a gene encoding dihydroxyphenylalanine (DOPA) decarboxylase involved in dopamine and serotonin biosynthesis. In contrast, genes related to antioxidant defense and metabolism showed minimal changes. **Conclusion:** Social interaction with younger individuals extends lifespan in aged male *D. melanogaster* independent of vitamin C supplementation. These findings suggest may exert a longevity-promoting effect that is not replicated by antioxidant intervention and may involve adaptive, non-antioxidant pathways potentially linked to neurotransmitter-related mechanisms.

Keywords: *Drosophila melanogaster*; social interaction; ascorbic acid; longevity; aging

1. Introduction

Aging is a complex, multifactorial process characterized by a transition from a relatively healthy physiological state to progressive functional decline driven by cumulative molecular and cellular damage, ultimately culminating in death [1]. These age-associated changes arise from the lifelong accumulation of damage induced by diverse stressors, originating from both physical and social exposures [2]. Such stressors promote the excessive generation of reactive oxygen species (ROS), leading to oxidative stress and subsequent damage across multiple tissues [3]. This process contributes to the development of numerous age-related diseases, including neurodegenerative disorders, cardiovascular disease, cancer, and chronic inflammatory conditions [4,5,6,7]. Consequently, the development of effective interventions to slow degenerative processes and reduce age-related morbidity remains a critical objective in aging research.

Despite extensive efforts, current intervention strategies aimed at modulating aging remain limited and often fail to account for substantial interindividual variability. Increasingly, integrative approaches that incorporate both nutritional and social determinants are recognized as necessary to achieve more comprehensive and robust outcomes. Vitamin C, a well-established antioxidant, has been shown to mitigate oxidative stress, thereby contributing to the prevention of age-related diseases and, in some contexts, to lifespan extension [8,9]. From a psychosocial perspective,

social support has likewise been associated with reduced cortisol levels, a key physiological biomarker of stress [10]. Although these factors originate from distinct biological and social domains, they converge on shared pathways of stress resilience and cellular damage attenuation that are central to the aging process.

Evaluating comprehensive interventions targeting aging presents substantial methodological challenges in human populations due to ethical constraints, high costs, and biological complexity. Consequently, suitable model organisms are essential for investigating these processes under controlled experimental conditions. *Drosophila melanogaster* represents a particularly powerful system, owing to its low maintenance cost, short life cycle, and the conservation of approximately 75% of human disease-related genes [11]. These features facilitate efficient investigation of both long-term and multigenerational effects. Moreover, *D. melanogaster* has been extensively validated as a robust model for evaluating anti-aging interventions and lifespan-extending compounds [12,13,14,15,16], making it especially suitable for studying the combined effects of nutritional and social strategies.

Vitamin C supplementation in *D. melanogaster* has been reported to extend lifespan, primarily through its capacity to reduce oxidative stress and improve metabolic efficiency [17,18]. Although the molecular consequences of vitamin C supplementation may vary across species, its fundamental role in modulating oxidative stress represents a conserved mechanism relevant to aging across biological systems. In more complex organisms, particularly humans, vitamin C has been associated with the maintenance of telomere length, an effect generally attributed to its ability to suppress oxidative damage and preserve genomic stability during aging [19]. Age-related declines in vitamin C levels have also been linked to increased susceptibility to cognitive impairment, potentially mediated by oxidative stress and disruption of neuronal homeostasis [20]. Beyond nutritional influences, social factors play a critical role in shaping aging trajectories. Social engagement has been consistently associated with improved cognitive function, enhanced overall health, and a reduced risk of dementia, effects that are partly mediated through the attenuation of physiological stress responses [21,22,23]. In *D. melanogaster*, social interaction similarly modulates aging-related phenotypes; notably, exposure to younger conspecifics has been shown to enhance stress resistance and extend lifespan [24,25]. Despite these advances, the combined effects of nutritional antioxidants and social interaction on aging mechanisms remain insufficiently understood. In particular, it is unclear whether vitamin C supplementation and social interaction act synergistically or through independent pathways in influencing aging-related outcomes. Accordingly, this study aims to investigate the combined impact of vitamin C supplementation and social companionship on stress-related gene expression and lifespan in *D. melanogaster*. Given the global increase in life expectancy, such integrative approaches may provide accessible strategies to delay age-related decline and improve quality of life.

2. Materials and Methods

2.1 Fly Stocks

The experimental animals used in this study were fruit flies *D. melanogaster* of the *Oregon R(R)* strain, obtained from the Kyoto Drosophila Stock Center (Kyoto Institute of Technology, Kyoto, Japan). Flies were maintained in a controlled laboratory conditions at 25 °C with a 12:12 h light-dark cycle. All stocks were reared on standard cornmeal-based medium and maintained at a relative humidity of approximately 60–70%.

2.2 Fly Food and Compound Preparation

Standard fly food was prepared using cornmeal, yeast, agar, sucrose, propionic acid, and methyl paraben dissolved in water. Vitamin C was prepared as a 500 mM stock solution and added to the standard food to achieve final concentrations of 1.25 mM, 2.5 mM, and 5 mM. The selected concentrations were based on a previously published study [18]. However, preliminary optimization experiments indicated that higher concentrations of vitamin C exerted detrimental effects on the flies. Therefore, lower concentrations were chosen to maintain physiological relevance while minimizing potential toxicity.

2.3 Co-Housing Procedure and Treatment Vitamin C

Aged flies were obtained by maintaining *Oregon R(R)* males for 14 days and were subsequently designated as the aged group. Young flies consisted of *Oregon R(R)* females aged 1–3 days. To evaluate the combined effects of social interaction and vitamin C supplementation, aged males were co-housed with young females and provided with food containing vitamin C at the indicated concentrations (1.25 mM, 2.5 mM, and 5 mM) throughout the experimental period. Young females were replaced weekly to maintain a consistently young social environment. The experimental groups comparised an ‘old-old’ baseline control (natural aging without social intervension), an ‘old-young’ negative control without vitamin C (to evaluate social interaction effects), and ‘old-young’ treatment groups supplemented with vitamin C to evaluated the combined effects of social interaction and supplementation. During the sorting process, flies were randomly allocated to each experimental group without any selection preference for size or physical appearance. This experimental design was adapted from previously published protocols [24,25] with minor modifications. Notably, flies of the same genetic background were used for both aged and young groups to minimize potential genetic confounding. Aged males were specifically selected to avoid reproductive and physiological confounding factors associated with aging females [26].

2.4 Lifespan Assay

Flies were transferred to fresh food vials daily, and mortality was recorded each day until all aged flies had died. Each experimental condition consisted of six independent biological replicates, with 5 flies per vial (30 flies in total per condition). Lifespan data were plotted as survival curves, and statistical differences between groups were analyzed using the log-rank test.

2.5 Endurance Assay

After 14 days of co-housing, aged flies from each treatment group were transferred to vials containing 5 mL of standard medium. Ten aged flies per vial were subjected to the Power Tower apparatus to assess endurance over a 5-hour period. Each condition included three independent biological replicates. At hourly intervals, the number of flies that successfully climbed above a 2 cm threshold from the food surface was recorded. This assay was conducted following previously described protocols [27,28] with minor modifications, including adjustment of the climbing threshold to accommodate the apparatus dimensions and extension of the observation period to 5 hours. Data were analyzed using two-way analysis of variance (ANOVA) to evaluate the effects of treatment and time.

2.6 Locomotor Assay

Following the endurance assay, locomotor performance was assessed using the negative geotaxis assay. Aged flies were transferred to empty vials and gently tapped to position all individuals at the bottom. Ten flies per vial were used, with three independent biological replicates per condition. The proportion of flies that climbed beyond a height of 5 cm within 18 seconds was recorded as a measure of locomotor function [29]. Data were analyzed using one-way ANOVA.

2.7 Gene Expression Analysis

After 14 days of treatment, five aged flies from each group were pooled for RNA extraction using the Monarch® Spin RNA Isolation Kit (New England Biolabs, Ipswich, MA, USA), following the manufacturer's instructions. Gene expression analysis was performed using one-step RT-qPCR with the Luna® Universal One-Step RT-qPCR Kit (New England Biolabs, Ipswich, MA, USA) in a final reaction volume of 10 µL. The RT-qPCR protocol consisted of reverse transcription at 50 °C for 10 min, initial enzyme activation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Melt curve analysis was performed from 60 °C to 95 °C to confirm amplification specificity. Primer sequences are listed in Table 1, and *rp49* was used as the internal reference gene for normalization of target gene expression.

2.8 Data Analysis

All statistical analyses were performed using GraphPad Prism® 9 (GraphPad Software, San Diego, CA, USA). Lifespan data were presented as Kaplan–Meier survival curves and compared between groups using the log-rank test. Endurance data were analyzed using two-way ANOVA to assess the effects of treatment and time, followed by appropriate post hoc comparisons where applicable. Locomotor activity data were analyzed using one-way ANOVA. Gene expression data were analyzed using one-way ANOVA followed by Dunnett's post hoc test, with the control group used as the reference. Endurance, locomotor, and gene expression data are presented as mean ± standard deviation (SD). Statistical significance was defined as $p < 0.05$.

3. Results

3.1 Co-Housing With Young Individuals Extends Lifespan in Aged Individuals Without Additive Effects of Vitamin C

To investigate the effects of social interaction and vitamin C supplementation on aging-related phenotypes, *D. melanogaster* was used as an experimental model. Wild-type *Oregon R(R)* flies were maintained under standard laboratory conditions. For the co-housing experiments, 14-day-old males were designated as aged individuals, while 1–3-day-old females were used as young individuals. Co-housing was performed at a ratio of 1:2 (five aged males and ten young females) per vial without physical barriers, thereby allowing mating interactions to occur (Fig. 1A). A significant extension of lifespan was observed in aged males co-housed with young females compared with aged males maintained without young co-housing. In control conditions lacking young flies, population density was controlled by maintaining 15 individuals per vial (five aged males and ten aged females). To determine whether vitamin C supplementation modulated this effect, co-housed flies were provided with food supplemented with vitamin C at concentrations of 1.25 mM, 2.5 mM, or 5 mM. No significant differences in lifespan were detected among co-housed groups receiving vitamin C compared with those without supplementation (Fig. 1B), indicating that vitamin C did not further enhance the lifespan extension induced by co-housing with young individuals.

To assess whether social interaction-induced lifespan extension was accompanied by changes in functional performance, endurance was evaluated using a 5-hour Power Tower assay. A significant difference was observed at the second hour of testing, with aged flies co-housed with young individuals exhibiting greater endurance than aged flies without young co-housing (Fig. 1C). However, no significant differences were detected at later time points. Notably, aged flies co-housed with young individuals and supplemented with vitamin C at 2.5 mM or 5 mM displayed a marked and progressive decline in endurance over the course of the assay. Locomotor performance was subse-

Table 1. Primer sequences used for real-time qPCR.

Genes	Forward primer	Reverse primer
rp49	CGCTTCAAGGGACAGTATCTG	AAACGCGGTTCTGCATGAG
dpt	GCTGCGCAATCGCTTCTACT	TGGTGGAGTGGGCTTCATG
totA	CCAAAATGAATTCTTCAACTGCT	GAATAGCCCATGCATAGAGGAC
sod1	AGGTCAACATCACCGACTCC	GTTGACTTGCTCAGCTCGTG
sod2	TGGCCACATCAACCACAC	TTCCACTGCGACTCGATG
srl	CTCTTGGAGTCCGAGATCCGCAA	GGGACCGCGAGCTGATGGTT
tom40	TGCACGTGTGCTACTACCAG	ATTCCGCCTCTGAAGACCAG
ddc	GGGTTTGATTCCCTTCTACGC	CGTGGGGAAGTAGGCATGAAA

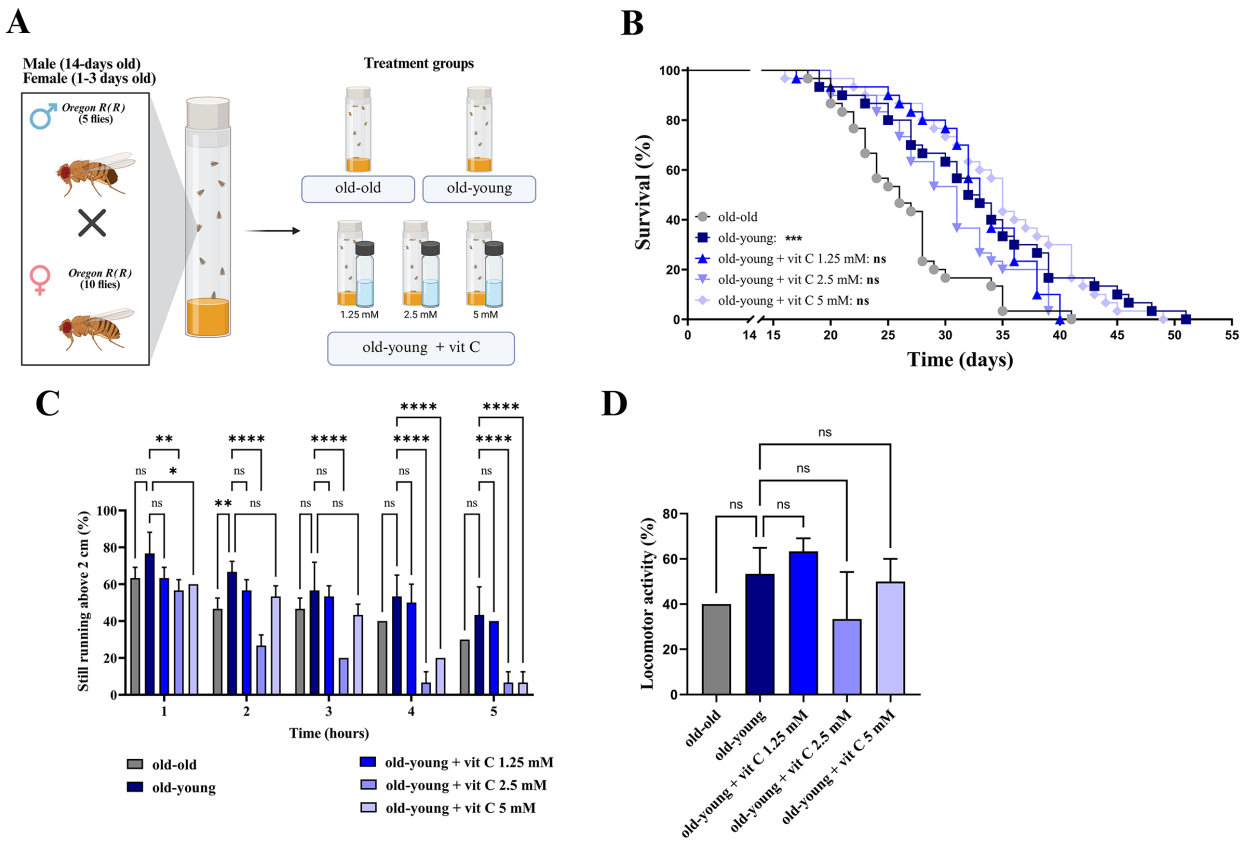

Fig. 1. Co-housing with young individuals extends lifespan in aged flies, whereas vitamin C supplementation does not confer additional longevity benefits. (A) Schematic illustration of the experimental design for co-housing aged and young flies with vitamin C supplementation. (B) Kaplan–Meier survival curves of aged *Oregon R(R)* males (14 days old) co-housed with young females (1–3 days old) and supplemented with vitamin C at 1.25 mM, 2.5 mM, or 5 mM. (C) Endurance performance after 5 hours of training of aged flies co-housed with young flies with or without vitamin C supplementation, expressed as the percentage of flies still running above 2 cm during the endurance assay. (D) Locomotor activity post 5 hours endurance training of aged flies co-housed with young flies with or without vitamin C supplementation, expressed as the percentage of flies reaching the defined climbing threshold. ns, non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$ (n = 30).

quently assessed using a negative geotaxis assay following the endurance test. No significant differences were observed among the treatment groups, indicating that neither co-housing with young individuals nor vitamin C supplementation significantly affected locomotor activity in aged flies (Fig. 1D).

3.2 Co-Housing With Young Individuals and Vitamin C Treatment Modulate Stress Response–Related Gene Expression in Aged Individuals

To further elucidate the mechanisms underlying lifespan extension in aged flies co-housed with young individuals, we examined stress-response pathways that have previously been implicated in aging and longevity [24,30].

Given that both social interaction and antioxidant supplementation have been associated with stress modulation, we assessed whether exposure to young flies and vitamin C treatment altered stress-related gene expression in aged flies relative to those maintained without young co-housing.

We first evaluated the expression of *dpt* (*diptericin*), an antimicrobial peptide gene commonly used as a readout of innate immune activation in *Drosophila*. Beyond infection, *dpt* expression can also be induced by non-infectious stress through growth-blocking peptide (GBP)–mediated signaling [31]. Aged flies co-housed with young individuals exhibited significantly lower *dpt* expression compared with aged flies maintained without young co-housing (Fig. 2A), suggesting that exposure to younger individuals may attenuate immune or stress-related activation. Interestingly, vitamin C supplementation resulted in increased *dpt* expression relative to the non-supplemented co-housed group. Although *dpt* induction is typically associated with immune activation or stress, the absence of a corresponding reduction in lifespan suggests that this increase does not necessarily reflect detrimental physiological effects in this context.

We next examined the expression of *totA* (encoded for Turandot A), a well-established marker of systemic stress responses in *Drosophila*. Aged flies co-housed with young individuals displayed significantly elevated *totA* expression compared with aged flies without young co-housing (Fig. 2B). A further increase in *totA* expression was observed in co-housed flies receiving vitamin C supplementation. Elevated *totA* expression is commonly associated with adaptive stress responses and has been linked to stress tolerance rather than pathological damage, consistent with a hormetic response.

To determine whether the observed changes in *dpt* and *totA* expression reflected altered oxidative stress status, we analyzed the expression of endogenous antioxidant genes *sod1* and *sod2*, which encode cytosolic and mitochondrial superoxide dismutases, respectively [32]. No significant differences in *sod1* expression were detected among any experimental groups, including aged flies without co-housing, aged flies co-housed with young individuals, or co-housed flies treated with vitamin C (Fig. 2C). This finding supports the interpretation that the altered expression of *dpt* and *totA* does not reflect excessive oxidative stress. Similarly, *sod2* expression did not differ significantly between aged flies without co-housing and those co-housed with young individuals (Fig. 2D). A modest but significant reduction in *sod2* expression was observed only in the group treated with 1.25 mM vitamin C compared with the control, whereas no significant changes were detected at higher concentrations (2.5 mM and 5 mM).

3.3 Co-Housing With Young Individuals and Vitamin C Treatment Modulate the Expression of Metabolic and Neurotransmitter-Related Genes in Aged Individuals

The observation of controlled, non-toxic adaptive stress responses prompted further investigation into genes associated with metabolism and neurotransmitter synthesis that may contribute to lifespan regulation. Accordingly, we analyzed the expression of metabolic genes (*srl* and *tom40*) and the neurotransmitter-related gene *ddc* in aged flies under co-housing and vitamin C treatment conditions.

The *srl* gene encodes the *Drosophila* homolog of mammalian peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and functions as a key regulator of mitochondrial biogenesis and energy metabolism. Reduced *srl* expression has previously been associated with lifespan extension in *Drosophila* [33,34], making it a relevant target for this study. No significant differences in *srl* expression were observed between aged flies maintained without young co-housing and those co-housed with young individuals (Fig. 3A). Similarly, co-housed aged flies treated with 1.25 mM or 2.5 mM vitamin C did not exhibit significant changes in *srl* expression relative to untreated co-housed flies. In contrast, supplementation with 5 mM vitamin C resulted in a significant increase in *srl* expression. Despite this increase, no corresponding alteration in lifespan was observed, suggesting that elevated *srl* expression under these conditions does not adversely affect longevity.

We next examined *tom40*, a gene encoding a core component of the mitochondrial outer membrane translocase complex that is essential for mitochondrial protein import and cellular viability [35]. The expression of *tom40* did not differ significantly between aged flies without co-housing and those co-housed with young individuals (Fig. 3B). Likewise, no significant change was detected in co-housed flies treated with 5 mM vitamin C. However, co-housed aged flies treated with 1.25 mM and 2.5 mM vitamin C exhibited a significant reduction in *tom40* expression compared with untreated co-housed flies.

Given that the metabolic genes *srl* and *tom40* did not exhibit expression patterns consistent with the lifespan extension observed in aged flies co-housed with young individuals, we next investigated pathways more directly related to behavioral and social processes. We hypothesized that the observed longevity effects may be mediated, at least in part, by neurochemical pathways responsive to social context. To evaluate this possibility, we analyzed the expression of *ddc*, which encodes dihydroxyphenylalanine (DOPA) decarboxylase, a key enzyme in the biosynthesis of dopamine and serotonin. This gene has previously been implicated in natural variation in lifespan [36]. Our results show that aged flies co-housed with young individuals exhibited a significant upregulation of *ddc* expression compared with aged flies maintained without young co-housing (Fig. 3C). These findings suggest a potential link between

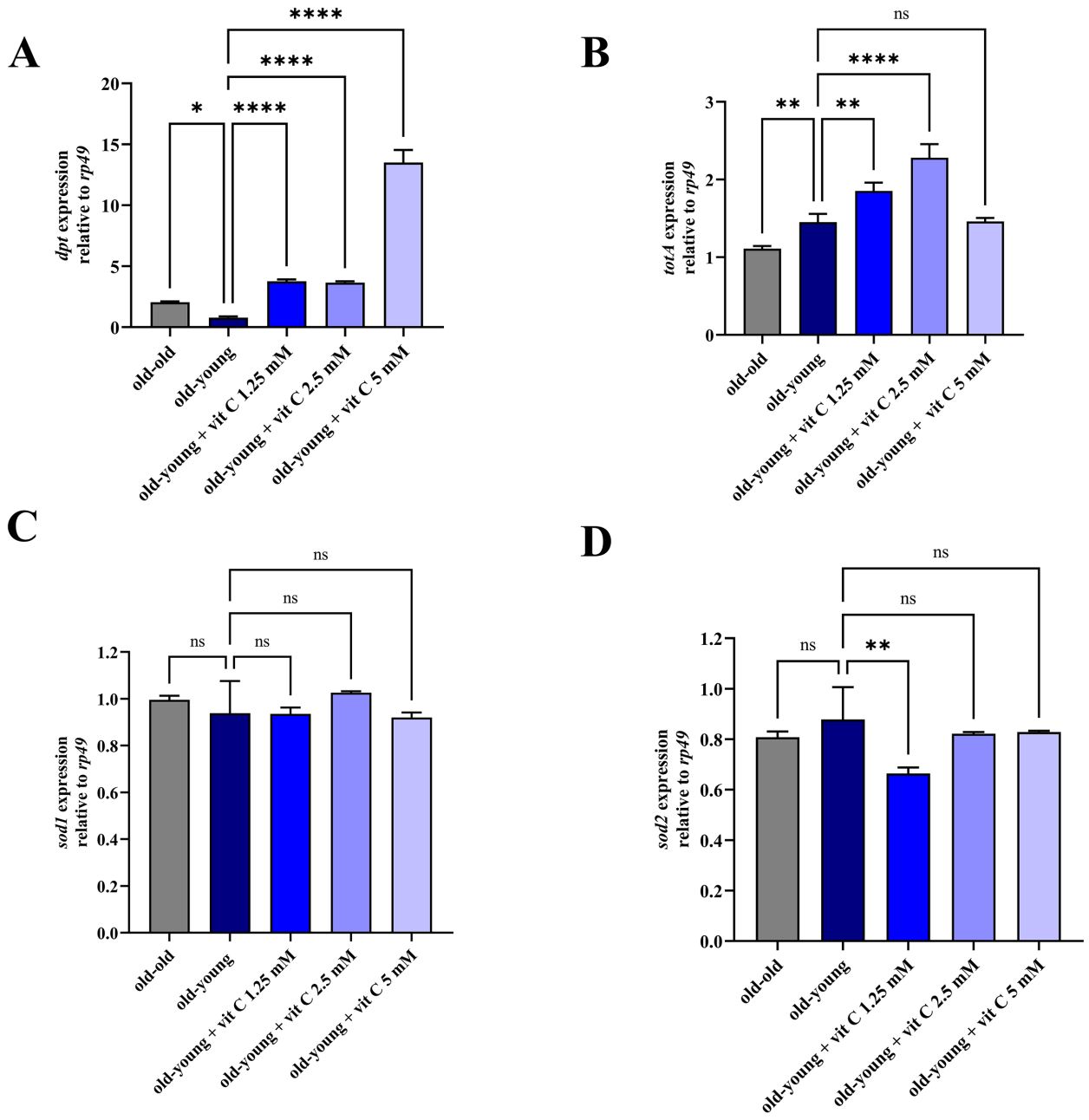

Fig. 2. Expression of stress response–related genes in aged *Oregon R(R)* male flies. Relative mRNA levels of (A) *dpt*, (B) *totA*, (C) *sod1*, and (D) *sod2* in old *Oregon R(R)* males housed alone (old-old), co-housed with young flies (old-young), or co-housed with young flies and supplemented with vitamin C at indicated concentrations (old-young + vit C). Data are shown as mean ± SD. ns, non-significant; *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$ (n = 3).

social interaction, modulation of neurotransmitter-related pathways, and lifespan extension, supporting the notion that neurobehavioral mechanisms may contribute to the beneficial effects of a young social environment.

4. Discussion

The present study demonstrates that social interaction with young female flies and vitamin C supplementation exert non-synergistic effects on aging phenotypes in male *D. melanogaster*. Notably, our findings reveal a clear dissociation between lifespan and physical performance. Co-housing aged males with young females significantly extended lifespan; however, the addition of vitamin C supplementation did not confer further benefit. Moreover, vitamin C failed to improve functional outcomes and, at certain concentrations, resulted in a reduction in endurance capacity. While vitamin C was initially expected to preserve endurance during repeated physical challenge, it instead pro-

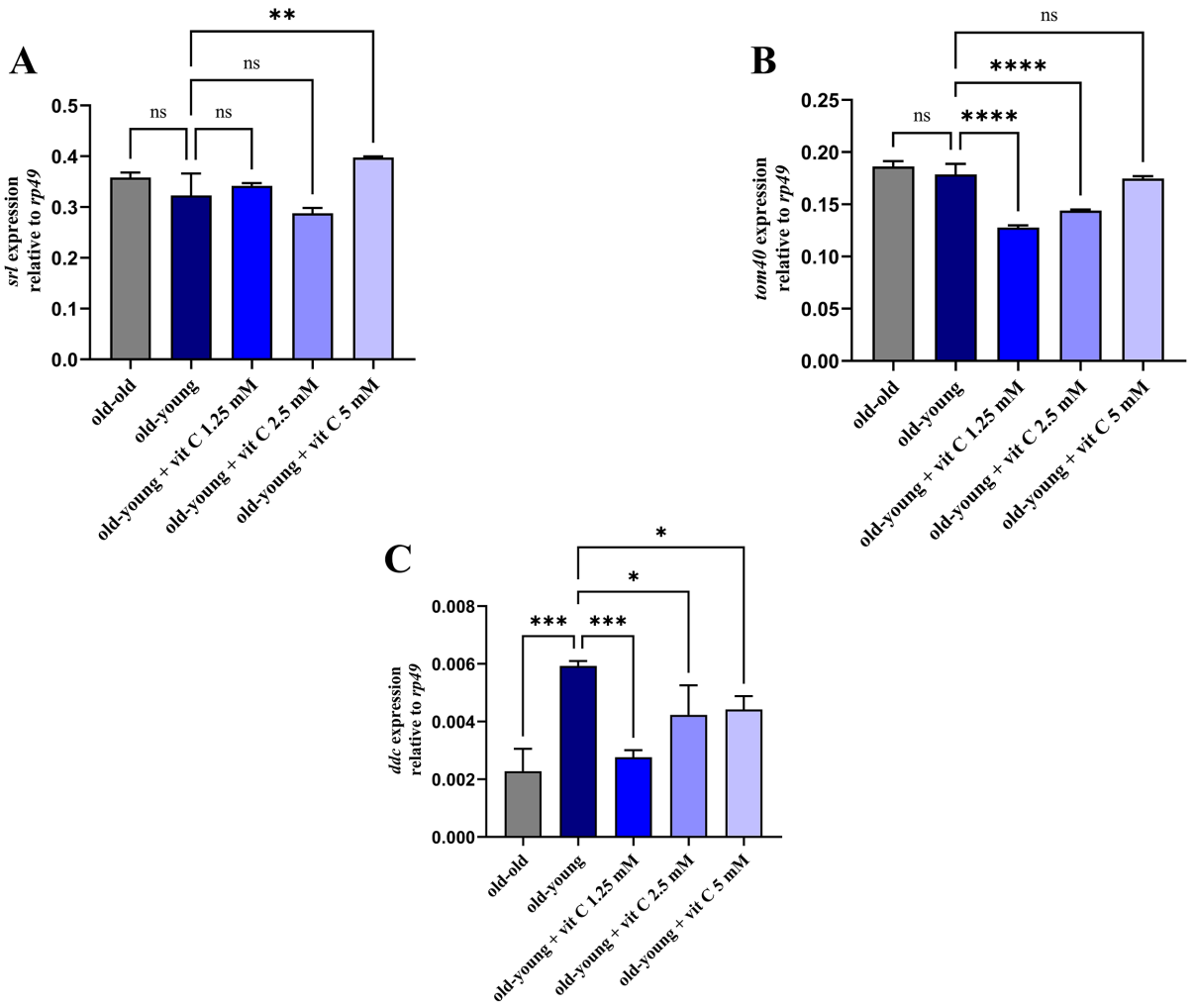

Fig. 3. Expression of metabolism- and neurotransmitter-related genes in aged Oregon *R(R)* male flies. Relative mRNA levels of (A) *srl*, (B) *tom40*, and (C) *ddc* in old *Oregon R(R)* males housed alone (old-old), co-housed with young flies (old-young), or co-housed with young flies and supplemented with vitamin C at indicated concentrations (old-young + vit C). Data are shown as mean ± SD. ns, non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$ (n = 3).

duced an adverse effect. In contrast, co-housing alone transiently improved endurance during the early phase of testing, although this benefit diminished over time. This pattern is consistent with a prior study in aged *Drosophila*, where physiological improvements are constrained by intrinsic limits of aging and decline with cumulative fatigue during prolonged stress exposure [37]. The negative impact of vitamin C on endurance observed in this study is consistent with previous findings in mammalian system, where supplementation has been associated with reduced mitochondrial protein content and decreased expression of genes involved in endurance adaptation [38]. These observations suggest that antioxidant supplementation may, under certain conditions, interfere with adaptive physiological responses to stress.

The observed increase in lifespan, particularly in the absence of improved physical performance, may be explained by differential activation of stress-response pathways. Social interaction with young females modulated the expression of *dpt*, a gene associated with immune activation and stress responses in aging flies. Notably, vitamin C supplementation induced upregulation of *dpt*, which may reflect activation of innate immune pathways [39,40]. However, given the concurrent reduction in endurance, the possibility that vitamin C exerts pro-oxidant effects cannot be excluded. Ascorbic acid can act as a pro-oxidant in the presence of transition metal ions such as iron and copper, promoting the generation of reactive oxygen species [41]. Consistent with this, a previous study has shown that vitamin C supplementation can exacerbate iron-induced toxicity in *Drosophila*, leading to reduced survival [18].

Further analysis of the systemic stress marker *totA* revealed increased expression in response to co-housing with young females, both with and without vitamin C supple-

mentation. This pattern is consistent with activation of an adaptive stress response. Importantly, in co-housed flies without vitamin C, this stress activation did not impair lifespan extension, suggesting a beneficial or hormetic effect. We hypothesize that this stress response represents a form of metabolic compensation in which the flies allocate energy toward maintaining long-term stress resistance, a strategy previously reported to correlate with longevity in *Drosophila* [42]. This condition, in turn, limits the energy available for high-intensity physical activity, in line with the resource allocation principle (trade-off) commonly found in insect biology [43]. In contrast, although vitamin C supplementation also induced stress-related gene expression, it did not confer lifespan benefits, indicating that the resulting stress response may be non-adaptive or maladaptive. This distinction highlights that not all stress pathway activation is beneficial; rather, the physiological context and mode of activation are critical determinants of outcome.

Evaluation of endogenous antioxidant defenses further supports this interpretation. Despite the recognized role of vitamin C as an exogenous antioxidant [44,45,46], neither social interaction nor supplementation significantly altered *sod1* expression. Similarly, *sod2* expression remained largely unchanged across conditions, with the exception of a modest reduction at the lowest vitamin C concentration. This pattern of decrease appears to be opposite to the stress response shown by the *dpt* and *totA* genes, where an increase in *sod2* expression is expected as part of the antioxidant defense mechanism [47]. The lack of a clear dose-response relationship and inconsistent regulatory patterns in our findings suggest that *sod2* modulation may not be linear and is highly dependent on the fly's physiological conditions. The decrease in *sod2* expression at low concentrations of vitamin C may reflect a compensatory adjustment in which mitochondrial antioxidant defense is reduced in response to a decrease in oxidative load [48]. Overall, the lack of consistent changes in antioxidant gene expression indicates that enzymatic antioxidant defenses are unlikely to be the primary drivers of the observed lifespan extension.

Analysis of metabolic and mitochondrial-related genes (*srl* and *tom40*) further indicates that mitochondrial biogenesis pathways, including those associated with PGC-1α signaling, are not central to the observed effects. Although high-dose vitamin C increased *srl* expression and low-dose supplementation reduced *tom40* expression, these changes did not correlate with lifespan outcomes. In contrast, expression of *ddc* (encoded for DOPA decarboxylase) closely paralleled the lifespan extension phenotype. This gene has previously been implicated in natural variation in lifespan in *Drosophila* [36,49]. Although the role of DOPA decarboxylase has been extensively studied in the context of neurodegenerative disorder pathogenesis [50,51], its function in the physiological regulation of aging remains underexplored. The increased *ddc* expression observed in co-housed flies suggests that dopaminergic signaling may mediate the beneficial effects of social interaction. Elevated *ddc* expression is likely to enhance dopamine synthesis in the central nervous system, which is known to regulate courtship behavior, motivation, and social engagement in male flies [52,53]. Such changes may promote behavioral activation and adaptive responses that contribute to improved survival. Conversely, reduced *ddc* expression in vitamin C-supplemented flies may indicate disruption of dopaminergic signaling, potentially impairing neural plasticity and adaptive behavior [53,54]. Taken together, these findings support a model in which social interaction promotes longevity through neurobehavioral and adaptive stress-response pathways, whereas vitamin C supplementation, despite modulating similar molecular markers, fails to produce beneficial outcomes and may instead induce maladaptive physiological responses.

5. Limitations

This study has several limitations that should be considered when interpreting the findings. First, the molecular mechanisms distinguishing adaptive from maladaptive stress responses in *dpt* and *totA* gene expression remain incompletely characterized, limiting the ability to draw definitive causal inferences regarding their functional roles. Second, the physiological threshold at which antioxidant concentration in the diet begins to impair organismal fitness remains unclear, as a systematic concentration-response analysis was not performed and actual intake was not directly quantified. Third, the potential confounding influence of reproductive behavior on increased *ddc* expression and lifespan cannot be fully excluded, as courtship frequency and mating activity were not directly measured, nor were sterile fly models employed. This introduces uncertainty regarding the relative contribution of social interaction versus reproductive effort to the observed effects. Finally, although the findings suggest a possible role for *ddc* in mediating lifespan extension, definitive confirmation of this causal mechanism will require further validation using genetic approaches, such as *ddc* mutant lines.

6. Conclusions

This study demonstrates that co-housing aged male Drosophila melanogaster with young females significantly extends lifespan, whereas vitamin C supplementation does not confer comparable benefits and, at certain concentrations, negatively affects endurance capacity. The observed lifespan extension does not appear to be mediated by classical mechanisms such as enzymatic antioxidant defenses or mitochondrial biogenesis. In contrast to vitamin C supplementation, which was associated with reduced physical performance, social interaction with young individuals elicited an adaptive stress response that did not compromise overall vitality in aged males. Notably, the expression pattern of *ddc* (encoded for DOPA decarboxylase) consistently paralleled the lifespan extension phe-

notype, implicating dopaminergic signaling as a potential central mediator. These findings support the conclusion that social interaction induces neurochemical adaptations, particularly through increased *ddc* expression, which may enhance behavioral responsiveness and physiological resilience. This non-canonical, neurotransmitter-associated mechanism represents a primary driver of longevity in this context and highlights the capacity of social experience to modulate aging processes beyond traditional metabolic and antioxidant frameworks.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

AAis contributed to conceptualization, methodology, data curation, formal analysis, original draft preparation, review and editing, and visualization. EW contributed to conceptualization, methodology, data curation, formal analysis, original draft preparation, review and editing, and supervision. FDIN contributed to methodology and review and editing. NPL contributed to data curation, formal analysis, and review and editing. RD contributed to methodology and review and editing. AAsb contributed to data curation, formal analysis, and review and editing. WH contributed to methodology, data curation, formal analysis, and review and editing. MM contributed to data curation, formal analysis, and review and editing. HH contributed to methodology and review and editing. YR contributed to methodology and review and editing. FN contributed to conceptualization, methodology, data curation, formal analysis, original draft preparation, review and editing, and supervision. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work

Ethics Approval and Consent to Participate

Not applicable. The study was carried out in accordance with the ARRIVE guidelines.

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

The authors acknowledge the use of generative AI tools (ChatGPT) for language editing and grammar refinement during the preparation of this manuscript. No content generation, data analysis, or interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBE49064.

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