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Nutrition, Reproduction, Stress and Aging: Fifteen Years of our Research in *Drosophila*

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Abstract

Research using *Drosophila melanogaster* has provided fundamental insights into how diet, reproduction, genetics, and environment interact to shape aging. We showed that lifespan and reproductive traits are highly sensitive to nutritional balance, and flies represent an ideal system to disentangle these trade-offs. Across studies, protein-carbohydrate ratios, rather than caloric intake alone, determine whether investment favors reproduction or longevity. Flies naturally select nutrient ratios that maximize fecundity at the expense of survival, while dietary manipulations reveal sex-specific and transgenerational influences on stress resistance, metabolism, and antioxidant defenses. Developmental conditions, including larval diet, sugar identity, and crowding stress, further program adult physiology through insulin/IGF and TOR pathways. Our studies showed that insulin-like peptides provide non-redundant regulation of feeding, macronutrient allocation, and metabolic resilience, with neuromodulators and gut progenitors serving as key integrators of systemic signaling. Mitochondrial function and redox balance are important since expression of alternative dehydrogenases or modulation of CncC/Keap1 reshape stress resistance and lifespan. Studies of environmental toxicants, such as aluminum salts or nitric oxide donors, reveal how oxidative and nitrosative stress impair survival, while interventions like alpha-ketoglutarate or mild mitochondrial uncoupling confer context-dependent protection. Plant extracts, trace elements, and nanomaterials act as hormetic modulators of lifespan, although their benefits are tightly constrained by dose, sex, and diet. More than fifty studies published within fifteen years reveals that nutrition, reproduction, signaling pathways, and environmental exposures converge to determine healthspan, providing mechanistic insights with broad relevance to gerontology and translational biology.

Keywords: *Drosophila melanogaster*, nutrition, insulin/TOR signaling, reproduction, oxidative stress, lifespan

1. Introduction

For more than fifteen years, scientists for the Department of Biochemistry and Biotechnology of Carpathian National University used fruit fly *Drosophila* to explored how nutrition, reproduction, metabolism, and environmental stress shape the biology of aging. Using this model system, we have investigated the fundamental processes that determine lifespan and healthspan, focusing on the interactions between diet, physiological state, and conserved molecular pathways.

Our research integrated studies on macronutrient balance, insulin and target of rapamycin (TOR) signaling, mitochondrial function, oxidative stress, and the influence of environmental factors. Together, these investigations provide a systems-level view of how diet and physiology intersect with genetics to regulate reproduction, metabolic resilience, and longevity. By combining experimental genetics, nutritional interventions, and environmental challenges, we aim to uncover the principles that govern life-history trade-offs and adaptive responses. This work not only advances basic understanding of aging but also highlights the relevance of *Drosophila* as a model for human health, offering insights into diet–disease interactions, metabolic regulation, and the search for interventions that promote healthy lifespan.

2. Nutrition, Reproduction, and Aging

Research using *Drosophila* has provided key insights into the interactions between diet, reproduction, genetics, and aging. Because lifespan and reproductive traits are highly sensitive to both environmental and physiological factors, this model system is ideal for disentangling the complex trade-offs that underlie longevity.

Across studies, a consistent theme emerges that reproduction and lifespan represent competing outcomes of nutritional and physiological investment. Flies tend to regulate their diet toward protein-to-carbohydrate ratios that maximize reproduction, even though these choices reduce lifespan (Semaniuk et al. 2018a; Strilbytska et al. 2024). Interestingly, variation in food intake or egg production within a given diet does not predict longevity, emphasizing that macronutrient balance rather than quantity determines life-history outcomes. Moreover, reproduction and feeding behavior showed stronger associations with chronological than with biological age, underlining the importance of distinguishing between time-based and survival-based measures of aging (Lushchak et al. 2025).

Physiological condition further modulates these relationships. Differences between short- and long-lived strains reveal that elevated oxidative stress, altered mitochondrial function, and increased TOR and octopamine signaling are linked to shorter lifespan, whereas long-lived strains exhibit more stable metabolic profiles (Gubina et al. 2018). Mating status also exerts a strong influence, with virgin females living longer and polygamous individuals showing reduced lifespan alongside altered metabolism and antioxidant defense, pointing to insulin/insulin growth factor (IGF) and TOR pathways as mediators (Koliada et al. 2020). Interestingly, although oxidative damage accumulates with age, increased lipid peroxidation alone does not shorten lifespan, suggesting that damage to a single biomolecule class is insufficient to drive aging (Lushchak et al. 2016).

Together, these findings demonstrate that aging in *Drosophila* is shaped by an interplay between nutrient balance, reproductive effort, genetic background, and molecular pathways. Rather than a simple resource-based trade-off, lifespan and reproduction appear to be optimized by distinct nutritional and physiological regimes.

3. Macronutrient balance, carbohydrate quality, and protein intake

Nutrient composition, rather than calorie intake alone, is a critical determinant of lifespan, stress resistance, and metabolism. Studies using fruit fly have systematically examined how protein and carbohydrate levels, their ratios, and even the type of sugar consumed influence longevity, reproduction, stress tolerance, and metabolic enzyme activity.

Repeated exposure to diet with high-protein and low-carbohydrate content revealed fly adaptation over generations. Evolved lines showed increased survival by reducing food intake, accumulating storage metabolites such as glycogen, trehalose, and triacylglycerides, increasing alanine transaminase activity and urea levels, and reprogramming gene expression, including down-regulation of proteases and up-regulation of immune and storage proteins (Yurkevych et al. 2020).

Manipulating protein intake through yeast concentration showed that both protein restriction and excess shortened lifespan under stress, though high-protein diets improved heat resistance and enhanced antioxidant enzyme activities. High dietary protein also increased oxidative damage, with stronger effects in females (Strilbytska et al. 2021a).

Carbohydrate availability was also influential. High sucrose diets improved resistance to starvation, heat, cold, and oxidative stress, while low-sucrose diets induced mild oxidative stress signatures – including elevated lipid peroxidation and antioxidant enzyme activities (Strilbytska et al. 2022a). Moreover, sucrose consumption shortened lifespan by 13-27% and strongly reduced fecundity under low-protein conditions, whereas glucose and fructose produced less severe effects (Lushchak et al. 2014a).

Protein and carbohydrate balance was a stronger determinant of outcomes than calories alone. Diets with high protein-to-carbohydrate ratios shortened lifespan by increasing age-dependent mortality but improved fecundity and heat stress tolerance. Conversely, low protein diets extended lifespan at the expense of reproduction (Lushchak et al. 2012).

Using a nutritional geometry approach, flies provided with separate protein and carbohydrate sources exhibited different feeding behaviors compared with those restricted to mixed diets. Dietary choice led to elevated glucose and glycogen levels but reduced triglyceride stores. Regulation of trehalose and triglycerides varied substantially between choice and no-choice conditions, indicating that access to food options induces rapid metabolic reprogramming. These findings demonstrate that nutrient selection plays a crucial role in shaping carbohydrate and lipid metabolism and contributes to maintaining energy balance.

Using a nutritional geometry approach, we found that food choice significantly altered macronutrient intake, leading to higher glucose and glycogen levels but reduced triglyceride stores (Strilbytska et al. 2022b). The regulation of trehalose and triglycerides differed strongly between choice and no-choice feeding conditions, suggesting that dietary decisions trigger rapid metabolic reprogramming. The findings highlight the importance of nutrient selection in shaping carbohydrate and lipid metabolism, with potential implications for understanding metabolic regulation and disease. The study explores how dietary choice influences metabolism in *D. melanogaster*.

Parental diet also left transgenerational imprints. Offspring of flies reared on low-yeast diets showed persistent alterations in metabolic enzyme activity, including elevated lactate dehydrogenase (LDH) and altered malate dehydrogenase (MDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activity, indicating parental protein intake programs offspring metabolism (Strilbytska et al. 2022c).

Overall, these findings reveal that lifespan, fecundity, stress resistance, and oxidative balance are shaped not simply by calories but by the quality and balance of macronutrients. Protein promotes reproduction and acute stress resistance at the cost of longevity, while carbohydrate amount and type modulate both stress tolerance and reproductive output.

4. Parental Diet and Offspring Physiology

Parental nutrition strongly influences offspring physiology and metabolism in through non-genetic inheritance mechanisms (Vaiserman and Lushchak 2019a; 2021). Altered protein or carbohydrate levels in parental diets affect stress resistance, antioxidant defenses, and metabolism in a sex-specific manner.

Low protein in the parental diet reduced cold tolerance in male offspring and altered activities of antioxidant enzymes such as superoxide dismutase and catalase. Males also showed changes in second-line antioxidant enzymes and thiol levels, while females were largely unaffected. These results suggest that protein-dependent modifications in parental diets can reprogram oxidative stress responses across generations (Strilbytska et al. 2021b, 2022).

Carbohydrate variation also showed clear transgenerational effects. Offspring from parents fed low-sucrose died had elevated activities of key metabolic enzymes (LDH, MDH, ALT) and

higher urea content, indicating intensified carbohydrate and amino acid metabolism. In addition, antioxidant enzyme activities increased in males under low parental sucrose, but females showed the opposite trend with higher sucrose. Levels of thiol compounds also varied depending on sex and parental carbohydrate supply (Strilbytska et al. 2021c).

These findings highlight that both protein and carbohydrate content in parental diets shape offspring metabolic and antioxidant status in a sex-dependent way. Such transgenerational effects underline the importance of parental nutrition in defining stress resistance, energy balance, and longevity-related traits in *Drosophila*.

5. Developmental diet, carbohydrate quality, and stress sensitivity

Studies in flies has shown that early-life nutrition, sugar type and amount, and developmental stress leave lasting effects on metabolism, oxidative state, and lifespan. The following studies examine how developmental diet, glucose and fructose availability, sucrose levels, and larval crowding interact with mitochondrial function, antioxidant defenses, and life-history traits to shape adult physiology and aging.

Developmental diet has long-term consequences for metabolism and metabolic complications (Vaiserman et al. 2018; Vaiserman and Lushchak 2019b). The macronutrient environment during larval stages partially determines adult hemolymph glucose, glycogen, triacylglycerides, and body composition across different ages, suggesting that insulin/TOR signaling mediates how early nutrition programs later-life metabolic patterns (Stefanyshyn et al. 2023).

The identity and dose of sugars strongly influence metabolic outcomes. High fructose intake promoted obesity-like phenotypes, with elevated carbohydrate and lipid storage, higher uric acid, increased feeding, and altered insulin-like peptide expression, while high glucose intake led to stronger hyperglycemia and developmental toxicity (Rovenko et al. 2015a). Feeding larvae glucose or fructose produced distinct oxidative-stress signatures and antioxidant enzyme profiles in adults, with notable sex differences (Lushchak et al. 2011). Interestingly, restriction of either glucose or fructose induced mild oxidative stress independent of mitochondrial ROS production, implying that limited sugar availability can trigger adaptive stress responses (Rovenko et al. 2015b).

Sucrose intake also had a dual impact. Excessive sucrose during development induced obesity-like traits with higher lipid and glycogen levels, while very low sucrose under protein-rich conditions caused oxidative stress and upregulation of antioxidant defenses, indicating that both excess and scarcity of sugar can impair physiology, but via different pathways (Rovenko et al. 2015c).

Non-nutritional developmental stressors such as larval crowding showed hormetic effects. Flies that eclosed early under crowded conditions lived longer, with increases in mean and maximum lifespan, and displayed altered expression of longevity-related genes such as *InR, Hsp70, dSir2, dTOR*, and *dFOXO* in a sex-specific manner (Lushchak et al. 2018). These findings indicate that moderate developmental stress can "prime" flies for longevity, while severe stress reduces fitness.

Altogether, these studies demonstrate that developmental diet and environment strongly condition adult metabolic trajectories. Carbohydrate type and amount influence whether sugars act as energy sources, obesogens, or mild stressors, while crowding stress can extend lifespan through hormetic responses. Insulin/IGF and TOR signaling, antioxidant defenses, and mitochondrial adjustments consistently emerge as key mediators of these effects.

6. Insulin-like peptides and nutrient-dependent feeding and metabolism

Drosophila insulin-like peptides (DILPs) are central regulators of growth, carbohydrate and lipid metabolism, feeding behaviour, stress resistance and lifespan (Fig. 1). Because flies express multiple DILPs with partly distinct expression patterns and functions, genetic manipulation of individual dilp genes combined with controlled dietary challenges allows dissection of how specific DILPs shape appetite, macronutrient choice and storage metabolism.

Genetic loss of particular DILPs changes both feeding preference and metabolic allocation in a diet-dependent manner. Mutants lacking *dilp2*, *dilp3*, *dilp5* or *dilp7* alter macronutrient choice and show compensatory changes in protein versus carbohydrate consumption; *dilp2* mutants, for example, display reduced glycogen, whereas *dilp3* deficiency elevates circulating trehalose and glycogen under low-protein conditions (Semaniuk et al. 2018b). These data imply that individual DILPs contribute non-redundantly to balancing storage and circulating carbohydrates and to setting appetitive responses to diet.

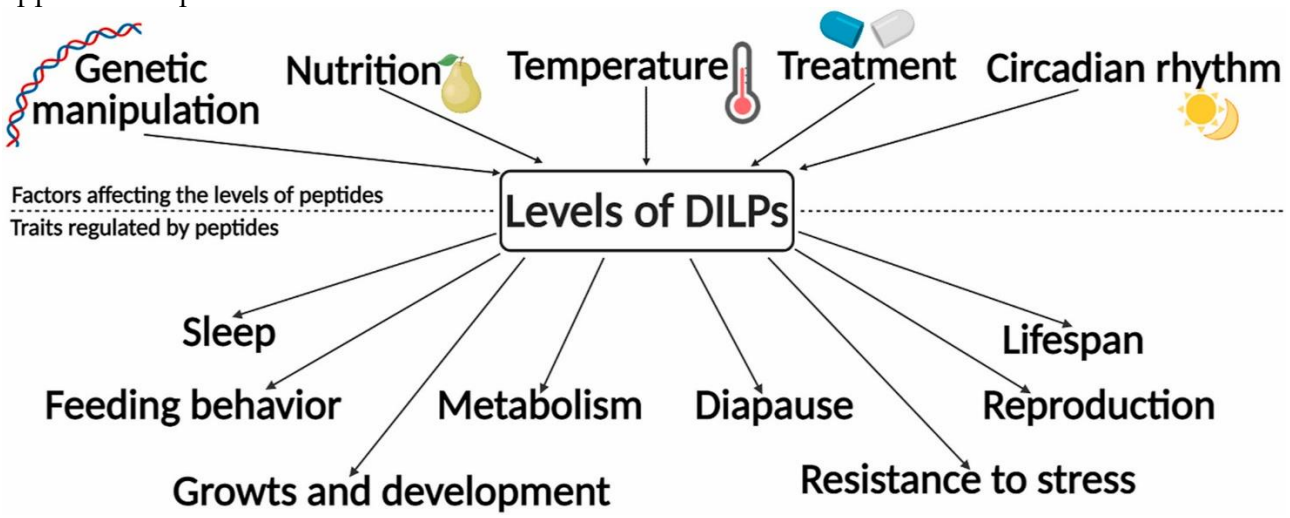

Figure 1. Determinants of insulin-like peptide expression patterns and their links to physiological and metabolic traits in *Drosophila*. Nutrition, dietary supplements and environmental factors affect the expression of DILPs. *Drosophila* Insulin-like peptides regulate lifespan, reproduction, development, feeding behaviour and metabolism. From Semaniuk et al. 2021c.

Follow-up work demonstrated that DILPs also control concentration-dependent appetitive responses to different sugars. Wild-type flies regulate carbohydrate intake by "compensatory feeding" (ingesting less volume as sugar concentration rises), but mutants in several *dilp* genes lose or weaken that compensation for glucose and fructose and often consume larger amounts of sucrose at moderate–high concentrations (Semaniuk et al. 2021a). Thus, DILPs shape not only what flies eat (protein vs carbohydrate) but also how appetite scales with carbohydrate identity and concentration, with different DILPs more important for responses to glucose, fructose or sucrose.

A broader synthesis and review of DILP biology places these experimental results in context: DILPs 1-7 largely signal via the single *Drosophila* insulin receptor to activate conserved IIS/PI3K–Akt–FOXO circuitry, while DILP6 and DILP8 have somewhat specialized roles (IGF-like and relaxin-like functions, respectively). The review emphasizes that DILPs act in a distributed, tissue-specific manner (brain IPCs, gut, fat body), are regulated by nutrient state and cross-talk with other hormonal and stress pathways, and that modulation of DILP signaling is a major lever by which diet and environment influence lifespan and metabolic health (Semaniuk et al. 2021b).

Mechanistically, the combined studies show three consistent points. First, different DILPs have specific roles in nutrient sensing and storage regulation (e.g., *dilp5* loss lowers triglycerides and glycogen; *dilp3* loss elevates circulating sugars) (Semaniuk et al. 2018b). Second, appetite regulation by DILPs depends on carbohydrate identity and concentration — mutants vary in their compensatory feeding to glucose, fructose and sucrose, implying distinct neural/hormonal pathways for different sugars (Semaniuk et al. 2021a). Third, the distribution and context-dependent secretion of DILPs (brain vs gut vs fat body), together with interaction with TOR and other pathways, explain how local peptide signals scale to systemic metabolic outcomes and life-history effects (Semaniuk et al. 2021c)

Collectively, these papers strengthen the view that the DILP family provides nuanced, peptide-specific control of feeding behavior and metabolic partitioning, and that manipulating individual DILPs or their dietary modulators offers a tractable route to probe mechanisms linking nutrient environment to metabolic disease and aging.

7. Role of insulin and TOR pathways in the gut

The gut is increasingly recognized as a key regulator of organismal physiology, integrating nutrient signals and coordinating systemic responses via conserved signaling pathways. In *D. melanogaster*, intestinal stem and progenitor cells (ISCs and EBs) are essential for tissue homeostasis and longevity. Recent studies highlight how insulin and TOR pathways in these cells influence metabolism, stress resistance, and lifespan.

Insulin signaling within intestinal progenitors is a major determinant of metabolic and aging traits. Upregulation of insulin receptor (InR) signaling in these cells improved stress resistance and survival under dietary restriction, while downregulation shortened lifespan and impaired metabolism. The findings highlight that local gut insulin signaling not only regulates ISC proliferation but also exerts systemic control over carbohydrate and lipid balance (Strilbytska et al. 2019, 2021d).

The TOR pathway exerts complex, context-dependent effects. Inhibition of TOR signaling in intestinal stem and progenitor cells led to shorter lifespan on both standard and malnourished diets, as well as reduced stress resistance under starvation or oxidative challenge. TOR knockdown decreased glycogen and triacylglyceride stores and altered expression of insulin-like peptide genes and JAK/STAT cytokines, indicating broad systemic metabolic effects (Strilbytska et al. 2020a).

Conversely, activation of the TOR–Myc axis by overexpressing Rheb or Myc-Rheb in midgut progenitors reduced lifespan and starvation resistance but improved survival under malnutrition. TOR/Myc activation also disrupted carbohydrate metabolism, activated JAK/STAT and insulin signaling locally, and impaired gut barrier function (Strilbytska et al. 2017). These results suggest that while TOR activity supports growth and nutrient handling, chronic hyperactivation accelerates aging.

More broadly, these findings support the role of the gut as a regulatory hub. Insulin and TOR signaling in intestinal progenitors dictate systemic energy allocation, stress response, and longevity. The balance between sufficient proliferative/metabolic signaling and avoidance of overactivation is critical for organismal health.

8. Neuromodulatory regulation of feeding, stress and metabolism

Drosophila provides a compact neural and endocrine system for studying how sensory cues and neuromodulators control feeding, metabolism and stress responses. Recent work shows that olfactory cues, monoamines and peptidergic neurons all converge on the insulin-producing cells (IPCs) to coordinate anticipatory endocrine responses and longer-term metabolic state.

Acute exposure to attractive food odors such as apple-cider vinegar or life fermenting yeasts triggers a rapid, transient increase in circulating glucose together with quick transcriptional upregulation of adipokinetic hormone (AKH) and several insulin-like peptides (DILPs). By contrast, sustained odor exposure reduces food intake and produces steady changes in metabolic gene expression. These odor-driven responses link olfactory sensitivity to endocrine signaling and carbohydrate/lipid balance (Lushchak et al. 2015).

Serotonin and octopamine act via distinct receptors expressed in IPCs and produce different physiological outputs. Manipulating the serotonin receptor 5-HT1A versus the octopamine receptor OAMB in IPCs changes DILP transcription, carbohydrate stores, starvation resistance, food intake and aspects of social behavior in opposite ways. These results show that different aminergic pathways tune IPC activity and downstream metabolism and behavior (Luo et al. 2014).

A defined group of dorsal lateral peptidergic neurons co-expressing short neuropeptide F (sNPF) and corazonin (CRZ) projects onto IPCs. Knockdown of these peptides alters Dilp transcription, starvation resistance and carbohydrate/lipid metabolism, indicating that co-released peptides from a compact circuit coordinate energy balance and stress resilience (Kapan et al. 2012).

Together, these studies show that sensory inputs (food odors) and multiple neuromodulatory systems (monoamines, peptidergic neurons) converge on IPCs to produce both rapid anticipatory endocrine changes and longer-term shifts in metabolism, feeding and stress resistance. The outcome depends on stimulus duration and the specific neuromodulatory pathway engaged, which allows flexible regulation of energy balance.

9. Mitochondrial function and redox signaling as determinants of lifespan

Mitochondrial function and redox signaling are central to lifespan regulation. *Drosophila melanogaster* has been used to test interventions that bypass complex I of the respiratory chain and to study genetic regulators of antioxidant responses. Three key studies highlight how alternative NADH dehydrogenases and the CncC/Keap1 pathway alter stress resistance, metabolism, and longevity.

Alternative NADH dehydrogenase (aNDH) from the *Ciona intestinalis* was expressed in *Drosophila*. Unlike the conventional complex I of the mitochondrial respiratory chain, aNDH is a single-subunit enzyme that transfers electrons from NADH to ubiquinone without pumping protons across the inner membrane. Authors explored its biochemical properties, genetic structure, and evolutionary significance, highlighting its potential role in bypassing complex I dysfunction and mitigating reactive oxygen species (ROS) production (Gospodaryov et al. 2014). The findings suggest that aNDH provides metabolic flexibility and stress resilience in organisms that possess it.

This research demonstrates that expression of *Ciona intestinalis* aNDH (NDX) in *Drosophila melanogaster* prolongs lifespan by 17–71% depending on diet, except under certain high-protein conditions where males lived shorter. NDX-expressing flies showed enhanced resistance to toxicants such as potassium iodate, alloxan, and 2,4-dichlorophenoxyacetic acid, though they were more sensitive to catechol and sodium chromate. Enzyme assays revealed increased glutathione S-transferase and glucose 6-phosphate dehydrogenase activities, pointing to activation of antioxidant and detoxification pathways. These results link aNDH expression to improved stress resistance and longevity, potentially mediated through Nrf2-regulated responses (Gospodaryov et al. 2020).

In contrast to these transgenic interventions, modulation of endogenous antioxidant pathways via the CncC/Keap1 system produced wide-ranging metabolic and stress-related effects. Flies with dKeap1 mutations, which enhance CncC activity, displayed elevated antioxidant capacity, reduced triacylglyceride content, and increased glucose and glycogen reserves. Conversely, flies with *cnc* loss-of-function mutants showed decreased mitochondrial respiration and reduced stress tolerance, though in some contexts they exhibited compensatory increases in antioxidant enzymes. Both genetic manipulations altered tolerance to starvation, oxidants, and temperature extremes in a sex-dependent manner (Bayliak et al. 2020).

Taken together, these studies reveal two complementary strategies for influencing aging and stress resistance in *Drosophila*. Introducing alternative dehydrogenases from yeast or tunicate species strengthens mitochondrial function and extends lifespan, while altering endogenous redox regulation through CncC/Keap1 reshapes energy storage and stress tolerance. Both highlight mitochondria and transcriptional stress responses as critical levers in the biology of aging.

10. Toxic Stress and Protection in *Drosophila melanogaster*

Fruit fly serves as a powerful model for studying the toxicity of environmental pollutants and chemical stressors, as well as for testing protective strategies. Our studies have focused on how compounds such as aluminum salts, sodium nitroprusside, ferrocyanide, and S-nitrosoglutathione

disrupt development and metabolism through oxidative and nitrosative stress, and how agents like alpha-ketoglutarate or mild mitochondrial uncouplers (e.g., 2,4-dinitrophenol) can provide partial protection.

Exposure to metals and nitric oxide donors consistently impaired development, reduced survival, and triggered oxidative and nitrosative stress in flies. Sodium nitroprusside and S-nitrosoglutathione delayed pupation, decreased adult emergence, and lowered activities of key enzymes such as aconitase and catalase, while simultaneously elevating markers of protein oxidation and increasing the activity of antioxidant enzymes such as superoxide dismutase and glutathione-S-transferase (Lozinsky et al. 2012; Lozinsky et al. 2013a). Similar stress responses were observed with ferrocyanide treatment, where toxicity was linked to the release of cyanide and iron ions (Lozinsky et al, 2013b).

Mitochondrial dysfunction and disrupted iron homeostasis emerged as central mechanisms of toxicity. Aluminum exposure, for instance, caused locomotor defects, decreased fecundity, and induced oxidative stress by displacing iron from proteins, increasing free iron levels, and impairing mitochondrial enzymes. Supplementation with alpha-ketoglutarate helped mitigate some of these effects by restoring metal balance, protecting aconitase activity, and improving survival, though it did not fully reverse behavioral impairments (Bayliak et al. 2019).

Protective strategies based on mitochondrial uncoupling were also tested. Low concentrations of 2,4-dinitrophenol alleviated some of the developmental delays and biochemical disturbances induced by sodium nitroprusside and ferrocyanide, restoring enzyme activities and reducing oxidative stress markers (Lozinsky et al. 2013a; Lozinsky et al. 2013b). These results support the concept that mild uncoupling can limit reactive oxygen species production and buffer against toxic stress, though protection was only partial.

Overall, the studies highlight that multiple environmental toxicants compromise *Drosophila* physiology through oxidative/nitrosative stress, impaired mitochondrial function, and disturbed iron metabolism, but also demonstrate that metabolic interventions such as alpha-ketoglutarate supplementation or mild uncoupling may provide effective, though incomplete, protection.

11. Context-Dependent Effects of Dietary Compounds and Stressors

Fruit fly remains one of the most powerful model organisms for investigating how nutrition, metabolism, and environmental factors shape health and lifespan. Its relatively short life cycle, genetic tractability, and conserved metabolic pathways make it particularly well suited for studying the balance between beneficial and detrimental effects of dietary interventions, supplements, and chemical exposures. Recent studies have explored a range of interventions, from supplementation with metabolic intermediates and amino acids to dietary macronutrient manipulations and exposure to novel compounds such as ethanol, mitochondrial uncouplers, and nanomaterials. These findings illustrate how *Drosophila* provides a versatile framework for identifying mechanisms of toxicity, protection, and lifespan regulation.

A central theme emerging across these studies is that dietary context critically shapes whether a compound exerts beneficial or harmful effects. Supplementation with alpha-ketoglutarate (AKG), a key intermediate in the tricarboxylic acid cycle, extended lifespan in long-lived *Drosophila* strains, while also modifying metabolic activity and stress responses (Demianchuk et al. 2024). This suggests that AKG acts not as a universal lifespan extender, but rather as a modulator that enhances metabolic resilience in already optimized physiological backgrounds. Its effects likely depend on cellular energy balance and redox state, offering parallels to findings in mammalian models where AKG supports mitochondrial health and reduces age-related decline.

Another amino acid, L-arginine, was shown to accelerate pupation and promote larger body size as well as higher reproductive output (Bayliak et al. 2017). Excessive intake of lard and fructose modulates pupation dynamics and compromises stress resistance mechanisms in adult *Drosophila*

(Hurza et al., 2022). These results underscore its role as a critical precursor in nitric oxide metabolism and protein synthesis. However, the increase in reproductive effort raises questions about possible trade-offs with longevity, consistent with the general life-history principle that reproduction and lifespan often compete for shared energetic resources.

Carbohydrate quality also had profound effects. Flies consuming high amylose starch diet developed obesity-like traits, including elevated lipid stores, altered insulin signaling, and reduced stress resistance (Abrat et al. 2020). This demonstrates that even within carbohydrate-rich diets, the structural properties of starch can dictate whether nutrients are stored beneficially or pathologically. Such findings provide a valuable model for studying diet-induced metabolic syndrome and obesity (Bayliak, 2020), conditions of increasing prevalence in humans.

The role of dietary ethanol was examined separately. Ethanol reduced lifespan and reproduction in a dose-dependent manner, while at the same time influencing oxidative stress markers and antioxidant defenses (Bayliak et al. 2022). Interestingly, low levels of ethanol in natural environments may serve as ecological cues or mild stressors, whereas chronic dietary exposure produces negative effects on both physiology and survival. These results highlight the duality of ethanol as both a potential signaling molecule and a metabolic toxin.

Pharmacological interventions provided further insights. The mitochondrial uncoupler 2,4-dinitrophenol (DNP) displayed a striking context dependence: it was toxic on low-caloric diets, where energy availability was already limited, but extended lifespan on nutrient-rich diets, where it induced mild uncoupling without overt metabolic disruption (Strilbytska et al. 2025). This dual action reinforces the idea that interventions targeting energy metabolism must be carefully balanced against nutritional environment, since even compounds with life-extending potential may become toxic under energy-limiting conditions.

Finally, novel environmental materials were also evaluated. Graphene oxide (GO), at low doses, produced hormetic effects in *Drosophila*, accelerating development, enhancing fecundity, extending lifespan, and improving resistance to stress, while lowering glucose and lipid stores (Strilbytska et al. 2022d). These results suggest that GO acts as a mild metabolic stressor, triggering protective responses that promote long-term resilience. At higher doses, however, graphene derivatives are known to be toxic, again demonstrating the fine line between beneficial stress and harmful exposure.

These findings reveal that interventions in *Drosophila* cannot be evaluated in isolation but must be understood in the context of diet composition, genetic background, and dose. Compounds such as AKG and DNP can enhance lifespan when conditions are favorable, whereas high amylose starch and ethanol clearly reduce survival and health. Graphene oxide illustrates the potential of nanomaterials to exert hormetic benefits, while also raising concerns about environmental safety. Overall, the studies highlight the dynamic interplay between nutrition, metabolism, and environmental exposures in shaping aging trajectories and health outcomes.

12. Plant-derived Compounds and Lifespan Modulation

Plant-derived supplements are increasingly investigated as modulators of lifespan and stress resistance in model organisms. *Drosophila melanogaster* is widely used to test such interventions due to its short lifespan, conserved metabolic pathways, and sensitivity to dietary phytochemicals. Recent studies have explored the effects of *Rhodiola rosea*, *Agastache foeniculum* (anise hyssop), and chili pepper on longevity, antioxidant defenses, and metabolic function.

Adaptogenic plants like *Rhodiola rosea* have been shown to exert mild stress-mimicking effects. Supplementation with *Rhodiola* extracts increased resistance to oxidative stress, improved survival under toxic exposures, and modulated activity of antioxidant enzymes in flies, supporting its role as a hormetic agent that enhances cellular defenses (Lushchak et al. 2014b). Similarly, dietary supplementation with *A. foeniculum* extracts extended lifespan and improved stress resistance. These effects were associated with changes in antioxidant system activity and metabolic regulation,

suggesting that polyphenolic compounds contribute to the protective outcomes (Strilbytska et al. 2020b).

Chili pepper supplementation provided more nuanced results. At low to moderate concentrations (0.04–0.12%), chili powder extended median lifespan by 9–13% in both sexes, reduced age-independent mortality, and improved cold shock resistance. It also lowered hemolymph glucose and altered metabolic enzyme activity in a sex-dependent manner, though high concentrations (3%) shortened male lifespan and reduced lipid stores (Semaniuk et al. 2022a). Another study focusing on antioxidant defenses showed that chili supplementation did not globally enhance enzymatic antioxidant capacity. Instead, female flies displayed a marked reduction in glutathione-S-transferase activity, while males at high chili concentration showed elevated superoxide dismutase activity. These findings indicate that chili's beneficial effects on lifespan are not mediated by classical antioxidant defense reinforcement but by subtler metabolic reprogramming (Semaniuk et al. 2022b).

Together, these studies highlight that plant-derived compounds can extend lifespan and improve stress resistance in *Drosophila*, but outcomes depend strongly on concentration, sex, and specific phytochemicals. Adaptogens like *Rhodiola* and *Agastache* generally act via hormetic upregulation of defenses, while chili pepper exerts a concentration-dependent dual effect - beneficial at low doses but harmful at high doses - through targeted metabolic modulation rather than broad antioxidant enhancement.

13. Insulin-Mimetic Effects and Oxidative Stress Induced by Chromium and Molybdenum

Trace elements such as chromium and molybdenum are increasingly studied for their potential roles in metabolic regulation and stress physiology. In mammals, these metals have been implicated in glucose metabolism and insulin signaling. *Drosophila melanogaster* offers a valuable model to investigate whether such effects are conserved, and how supplementation or exposure impacts development, oxidative balance, and metabolic pathways.

Chromium, administered as sodium chromate, demonstrated insulin-mimetic properties in flies. Supplementation lowered hemolymph glucose levels and altered carbohydrate and lipid metabolism, indicating enhanced peripheral glucose utilization (Perkhulyn et al. 2014). However, the effects were partial and context-dependent, suggesting chromium does not fully substitute for insulin-like signaling but can modulate metabolic outcomes in its presence.

Molybdate exposure produced two distinct patterns. First, sodium molybdate induced mild oxidative stress, reflected in increased levels of protein carbonyls and changes in antioxidant enzyme activity, without causing severe developmental toxicity (Perkhulyn et al. 2017). This response was characteristic of low-grade stress rather than acute toxicity. Second, molybdate partly mimicked insulin's metabolic actions by lowering glucose and modulating carbohydrate metabolism, though it also triggered signs of redox imbalance (Perkhulyn et al. 2017). Thus, like chromium, molybdenum influenced glucose regulation, but at the cost of mild oxidative stress.

Together, these findings suggest that sodium chromate and sodium molybdate act as metabolic modulators in *Drosophila*, partially reproducing insulin-like effects. Yet both elements carry trade-offs, as metabolic improvements are accompanied by redox disturbances. This duality mirrors the delicate balance of trace metals in physiology: essential in small amounts, but potentially disruptive if misregulated.

14. Sex, lipid biology and insulin signaling

Recent work in *Drosophila* emphasizes how sex, lipid handling and insulin signaling interact to determine lifespan, stress resistance and whole-body metabolism. The papers below (a mix of reviews and syntheses) clarify (1) sex-dependent responses to pro-longevity interventions, (2) conserved mechanisms of triacylglycerol (TAG) biology that connect nutrient status to health and

disease, and (3) how insulin-producing cells and insulin-like peptides (DILPs) are regulated by nutritional, neural and humoral cues.

Sex modifies the outcomes of anti-aging interventions: males and females often respond differently to dietary restriction, intermittent fasting, rapamycin and manipulations of insulin/TOR signaling (Fig. 2). The magnitude and direction of lifespan and healthspan effects depend on genetic background, mating status, dose and timing of the intervention, so sex must be treated as a key biological variable in gerontology studies (Lushchak et al. 2023).

TAG metabolism in *Drosophila* is largely conserved with mammals: fat-body lipid synthesis, storage in lipid droplets and regulated lipolysis govern energy buffering, starvation resistance and signals to other tissues. Hormonal regulators (DILPs, adipokinetic hormone) and nutrient-sensing pathways (TOR, AKH signaling and fat-body-derived factors) coordinate TAG turnover; dysfunction in these pathways recapitulates features of obesity and related pathologies in flies, making *Drosophila* a powerful model for TAG biology (Heier et al. 2021).

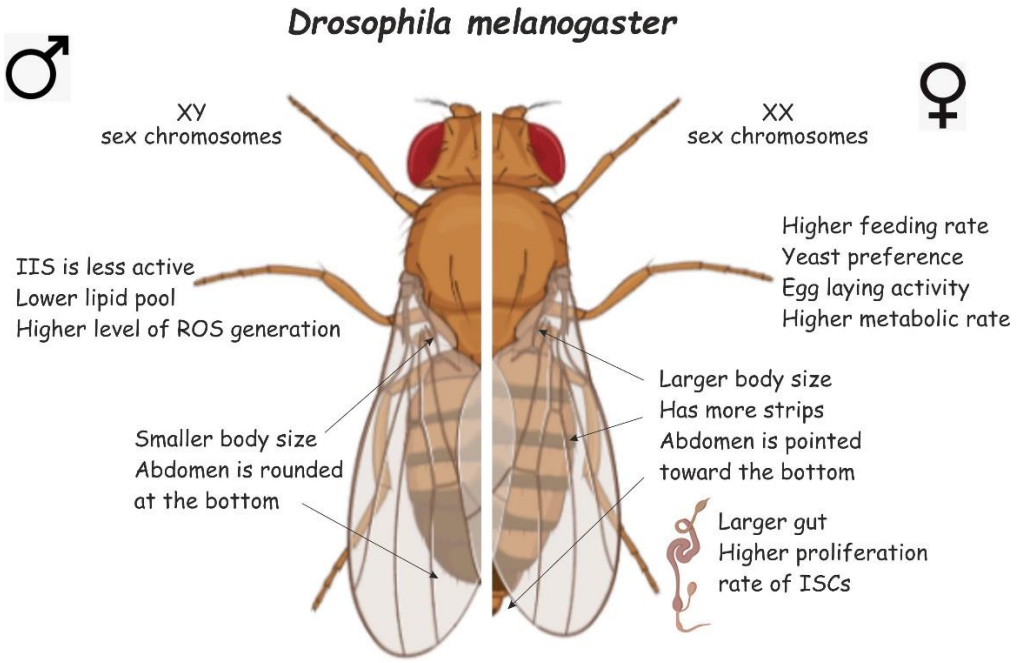

Figure 2. Sex-specific traits in *Drosophila*. Adapted from Lushchak et al., 2023.

DILP expression is highly plastic and responds to multiple inputs. Transcript levels of key brain DILPs (notably DILP2, DILP3, DILP5) change with protein:carbohydrate ratio, carbohydrate identity, starvation, temperature and pharmacological or natural supplements, and genetic manipulations in distant tissues can alter DILP transcription. These differential expression patterns help explain why diet, supplements and environment produce predictable shifts in feeding behaviour, storage metabolites and life-history traits (Semaniuk et al. 2021c).

At the cellular level, insulin-producing cells (IPCs) receive diverse regulatory inputs. IPCs integrate signals from the fat body (e.g., leptin-like Upd2, Dilp6), gut-derived peptides, and multiple neurotransmitters/neuropeptides (GABA, serotonin, octopamine, sNPF, corazonin), so that insulin release is coordinated with current nutrient state, stress and reproductive demands. This layered control allows local manipulations (cell- or tissue-specific) to scale to systemic metabolic effects (Nässel et al. 2013).

Together, these papers paint a coherent picture: sex and nutrient status shape systemic metabolism via TAG handling and finely tuned insulin signaling; at the same time, IPC regulation and DILP expression provide multiple nodes where diet, drugs or genetic changes can alter lifespan and stress resistance.

15. The sex of organ geometry

Laura Blackie and coauthors investigated whether internal organs have stereotypical three-dimensional arrangements that differ between the sexes in *Drosophila melanogaster*. Using high-throughput microCT imaging, they quantified organ shape, position, and adjacencies. The study revealed consistent but sexually dimorphic organ geometries: female guts were longer and more curved, while males had more tilted midgut loops. They also uncovered unexpected organ adjacencies e.g., gut proximity to testes in males versus ovaries in females, and left–right asymmetries. Importantly, the tracheal system, guided by sex-biased fibroblast growth factor signaling from gut muscles, actively maintains sex-specific intestinal shapes, showing that interorgan geometry is both dynamic and physiologically significant (Blackie et al. 2024).

16. Memory Enhancement by Ferulic Acid Ester Across Species

More than five years or years of identified a compound from *Rhodiola rosea*, ferulic acid eicosyl ester (FAE-20), as a potent memory enhancer across species. In *Drosophila*, supplementation with either crude extracts or synthetic FAE-20 improved larval associative memory and compensated for age-related decline in adults. In honeybees, *Rhodiola* extracts enhanced both memory acquisition and consolidation but not retrieval. In mice, FAE-20 increased neuronal excitability in hippocampal CA1 cells and improved contextual memory in both young and aged individuals. These findings suggest that FAE-20 may represent a conserved, plant-derived candidate for ameliorating memory impairments (Michels et al. 2018).

SUMMARY

Aging is a complex, multifactorial process shaped by interactions between genetics, physiology, and environment. Among environmental influences, nutrition and exposure to chemical stressors stand out as central regulators of lifespan, reproduction, and healthspan. The fruit fly *Drosophila melanogaster* has emerged as one of the most powerful models for dissecting these interactions, owing to its short lifespan, well-characterized genetics, conserved signaling pathways, and amenability to precise dietary manipulations. Over the past two decades, studies in *Drosophila* have revealed fundamental insights into how macronutrient balance, parental and developmental diet, insulin and TOR signaling, mitochondrial function, and exposure to natural or synthetic compounds shape the biology of aging.

One of the most consistent themes in nutritional gerontology is that lifespan and reproduction often represent competing outcomes of dietary allocation. Research in flies demonstrates that protein and carbohydrate balance, rather than caloric intake alone, is the primary determinant of longevity and fecundity. Flies tend to select protein-to-carbohydrate ratios that maximize reproduction, even when this shortens lifespan (Semaniuk et al. 2018a; Strilbytska et al. 2024). Manipulations of yeast or sucrose concentration alter stress resistance, oxidative damage, and metabolic enzyme activities in a sex-dependent manner (Strilbytska et al. 2021a, 2022a). Moreover, transgenerational studies reveal that parental diet programs offspring metabolism and antioxidant defenses, highlighting non-genetic inheritance of nutritional effects (Strilbytska et al. 2021b, 2022). Developmental environment also exerts long-lasting influence: larval diet composition, sugar identity, and crowding conditions shape adult body composition, oxidative state, and lifespan (Rovenko et al. 2015a,b,c; Lushchak et al. 2018). Together, these findings emphasize that nutrition at multiple life stages acts as both an immediate and a programming factor in determining health outcomes.

At the mechanistic level, conserved signaling pathways link nutrient availability to growth, metabolism, and longevity. The insulin/insulin-like growth factor (IIS) and target of rapamycin (TOR) pathways play central roles. *Drosophila* insulin-like peptides (DILPs) are secreted from specialized brain cells and peripheral tissues, and their expression is dynamically regulated by

dietary protein, carbohydrate type, neuromodulators, and hormonal cross-talk (Nässel et al. 2013; Semaniuk et al. 2021). Loss or overexpression of specific DILPs alters feeding behavior, carbohydrate and lipid metabolism, and stress tolerance, demonstrating non-redundant roles for individual peptides (Semaniuk et al. 2018b, 2021). IIS and TOR activity in the gut further exemplify how local nutrient signaling exerts systemic effects: manipulation of these pathways in intestinal progenitors modifies stress resistance, metabolic reserves, and lifespan (Strilbytska et al. 2017, 2020, 2021). Neuromodulators, including serotonin, octopamine, and short neuropeptide F, converge on insulin-producing cells to fine-tune peptide release according to environmental cues (Kapan et al. 2012; Luo et al. 2014). Collectively, these studies illustrate the multilayered regulatory architecture through which flies coordinate nutrient sensing with reproduction, stress physiology, and longevity.

Mitochondrial function and redox balance are equally important determinants of aging. Mitochondria are both sources and targets of reactive oxygen species, and interventions that stabilize mitochondrial activity can extend lifespan. Expression of alternative NADH from *Ciona intestinalis* allows bypass of complex I and confers resistance to xenobiotics, oxidative stress, and temperature extremes while extending lifespan (Gospodaryov et al. 2014, 2020). Endogenous stress pathways such as CncC/Keap1 further regulate antioxidant defenses, energy storage, and survival under starvation or toxic challenge (Bayliak et al. 2020). These findings point to mitochondria and redox signaling as central levers in aging biology, complementing the nutrient-sensing pathways described above.

Beyond endogenous mechanisms, *Drosophila* has been indispensable in toxicology and intervention studies. Flies exposed to environmental pollutants such as aluminum salts, ferrocyanide, sodium nitroprusside, and S-nitrosoglutathione display impaired development, reduced fecundity, and oxidative/nitrosative stress, largely mediated by mitochondrial dysfunction and disturbed iron homeostasis (Lozinsky et al. 2012, 2013a,b,c; Bayliak et al. 2019). Protective strategies have been explored: supplementation with alpha-ketoglutarate restores iron balance and partially rescues survival, while mild mitochondrial uncoupling by low-dose 2,4-dinitrophenol reduces reactive oxygen species production (Lozinsky et al. 2013a,b; Demianchuk et al. 2024). The outcomes of such interventions are strikingly context dependent - compounds that extend lifespan in nutrient-rich conditions may be harmful under dietary restriction, highlighting the importance of nutritional background in shaping pharmacological effects (Strilbytska et al. 2025).

Plant-derived compounds and trace elements provide additional perspectives. Adaptogenic herbs such as *Rhodiola rosea* and *Agastache foeniculum* act as mild stressors that upregulate antioxidant defenses and extend lifespan (Lushchak et al. 2014). Chili pepper supplementation shows concentration-dependent dual effects, improving lifespan and stress resistance at low doses but reducing survival at higher doses, through metabolic rather than classical antioxidant mechanisms (Semaniuk et al. 2022a,b). Trace elements including chromium and molybdenum partly mimic insulin signaling and modulate carbohydrate metabolism, though at the cost of mild oxidative stress (Perkhulyn et al. 2014, 2017; Rovenko et al. 2014). These findings reinforce the theme that interventions rarely have uniform effects; instead, benefits and risks depend on dose, sex, and metabolic context.

Sex has emerged as a critical variable across many of these studies. Males and females differ in their responses to dietary restriction, rapamycin, and IIS/TOR modulation, with sex-specific trade-offs between reproduction and longevity (Lushchak et al. 2023). Lipid metabolism is also sexually dimorphic, with conserved triacylglycerol pathways intersecting with hormonal and nutrient-sensing networks (Heier et al. 2021). Recent advances using high-resolution imaging have revealed that even internal organ geometry exhibits consistent sex-specific patterns: female guts are longer and more curved, while male midguts are more tilted, reflecting both developmental programming and ongoing regulation by tracheal signaling (Blackie et al. 2024). These insights highlight how sex differences extend beyond physiology to the very architecture of organ systems.

Finally, *Drosophila* studies also contribute to translational fields such as neurobiology and memory. A conserved compound from *Rhodiola rosea*, ferulic acid eicosyl ester, enhances memory across taxa including flies, honeybees, and mice by modulating neuronal excitability and compensating for age-related decline (Michels et al. 2018). This cross-species conservation underscores the relevance of fly research for identifying natural compounds with therapeutic potential in cognitive aging.

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Лущак ОВ (2025) Харчування, репродуктивна здатність, стрес і старіння: п'ятнадцять років наших досліджень на дрозофілах. *Журнал Карпатського національного університету імені Василя Стефаника. Біологія* 12: 117-135.

Дослідження з використанням *Drosophila melanogaster* надали фундаментальні уявлення про те, як взаємодія дієти, репродукції, генетики та довкілля формує процеси старіння. Наші результати продемонстрували, що тривалість життя та репродуктивні характеристики є надзвичайно чутливими до харчового балансу, а дрозофіла є оптимальною моделлю для розмежування цих компромісів. У різних дослідженнях показано, що співвідношення білків і вуглеводів, а не лише загальна калорійність, визначає, чи спрямовується ресурсний інвестиційний пріоритет на репродукцію чи на довголіття. Мухи природно обирають такі співвідношення поживних речовин, які максимізують плодючість ціною зниження виживаності, тоді як експериментальні маніпуляції з дієтою виявляють специфічні до статі та трансгенераційні впливи на стресостійкість, метаболізм та антиоксидантний захист. Умови розвитку, включно з личинковим харчуванням, типом споживаного цукру та стресом перенаселення, додатково програмують фізіологію дорослих особин через шляхи інсулін/IGF та TOR.

Наші дослідження показали, що інсуліноподібні пептиди здійснюють нереплікативну регуляцію харчової поведінки, розподілу макронутрієнтів і метаболічної стійкості, причому ключову інтегративну роль у системній сигналізації відіграють нейромодулятори та клітини-попередники кишечника. Функціонування мітохондрій та підтримка редокс-балансу є критично важливими, оскільки експресія альтернативних дегідрогеназ або модуляція системи CncC/Keap1 перебудовують стресостійкість та тривалість життя. Дослідження дії екологічних токсикантів, таких як солі алюмінію чи донори оксиду азоту, демонструють, що оксидативний та нітрозативний стрес знижують виживаність, тоді як втручання, зокрема α-кетоглутарат або помірне роз'єднання мітохондрій, забезпечують контекстно-залежний захисний ефект. Рослинні екстракти, мікроелементи та наноматеріали діють як герметичні модифікатори тривалості життя, хоча їх позитивні ефекти суворо обмежуються дозою, статтю та типом дієти.

Більше ніж п'ятдесят досліджень, опублікованих упродовж останніх п'ятнадцяти років, показують, що харчування, репродукція, сигнальні шляхи та впливи довкілля конвергують у визначенні тривалості здорового життя, забезпечуючи механістичні пояснення, що мають широку актуальність для геронтології та трансляційної біології.

Ключові слова: *Drosophila melanogaster*; харчування; сигнальні шляхи інсулін/TOR; репродуктивна функція; оксидативний стрес; тривалість життя