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Original Article

Undernutrition-induced stunting-like phenotype in *Drosophila melanogaster*

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Abstract

Stunting resulting from undernutrition is a significant global health challenge, particularly in developing countries, yet its underlying mechanisms and consequences remain inadequately understood. This study utilizes *Drosophila melanogaster* as an in vivo model to investigate the molecular basis of stunting. Due to the conserved nature of signaling pathways between *Drosophila* and vertebrates, this organism serves as an effective model for studying growth disorders. The aim of this study was to establish a *Drosophila* model exhibiting a stunting-like phenotype and to elucidate the molecular mechanisms underlying this condition. The stunting phenotype was induced through dietary manipulation, involving a standard nutrient-rich diet (100%) and treatment diets with reduced concentrations of sucrose, glucose, yeast, and cornmeal at 50%, 25%, and 12.5%. Phenotypic assessments included measurements of larval body size, fecundity, survival rates, and locomotor activity, alongside molecular analyses of gene expression related to metabolism, cell proliferation, and survival, using RT-qPCR. Results demonstrated that undernutrition profoundly affected *D. melanogaster*, causing growth retardation, reduced larval body size, diminished fecundity, and lower survival rates, though locomotor function remained unaffected. Molecular analysis revealed a significant decrease in the expression of the *totA* gene and notable increases in the expression of *dilp5*, *srl*, and *indy* genes, with no significant changes observed in the expression of the *pepck* gene. These findings indicate that undernutrition induces a stunting-like phenotype, likely driven by alterations in the expression of genes associated with metabolism, cell proliferation, and survival. Overall, this study establishes *D. melanogaster* as a valuable in vivo model for studying stunting-like phenotypes resulting from nutritional deficiencies and provides insights into the molecular pathways involved in growth impairment.

Keywords: *Drosophila*, stunting, phenotype, molecular, undernutrition

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Introduction

Nutritional deficiencies in children under five can lead to delayed development, with substantial long-term effects [1]. This can result in a substantial rise in both mortality and morbidity [2]. The most prevalent form of malnutrition is stunting, indicated by a height-for-age z-score that falls below minus two standard deviations [3], affecting approximately 162 million children under the age of five worldwide [4]. Stunting arises from inadequate nutritional intake combined with

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recurrent infections during the initial 1,000 days of life [5,6]. In developing countries, stunting represents a chronic nutritional challenge that impedes children's growth [1]. In Southeast Asia, approximately 25% of children under the age of five experience stunting [7]. According to data from the Asian Development Bank, Indonesia has the second-highest stunting rate among this age group in the region, with a prevalence that rose to 31.8% in 2020 [7]. Projections for 2025 suggest that stunting could result in growth faltering for 127 million children [4].

Many aspects of stunting remain unclear. Therefore, the use of animal models is essential for enhancing our understanding of the pathogenesis, physiological complexities, ecological factors, and genetic influences related to stunting [8,9]. However, the use of animal models in preclinical research, especially mammalian models, encounters challenges due to growing public concern regarding animal welfare and increased awareness of animal rights principles [10]. To address this issue, the use of alternative model organisms is recommended [10,11]. Substantial evidence demonstrates a strong relationship between growth and nutrition, and this association has been thoroughly investigated in various model organisms, including fruit flies (*Drosophila melanogaster*) [12], nematode worms (*Caenorhabditis elegans*) [13], zebrafish (*Danio rerio*) [14], mice (*Mus musculus*) [15], and rats (*Ratus norvegicus*) [16]. These studies have provided valuable insights into how nutrition influences fundamental biological processes. For example, studies utilizing *C. elegans* have demonstrated that developmental defects are linked to reduced nutrient availability [13]. Similarly, a study on zebrafish exposed to nutrient-deficient conditions revealed significant adverse effects on their survival, growth, and swimming capabilities [17]. In mammalian models such as mice, findings have indicated that inadequate nutrition leads to slowed growth and changes in the size of brain cells [18]. Furthermore, investigations on undernourished rats have revealed reduced weight gain [19]. Thus, exploring the impact of nutrition through the use of model organisms presents a compelling avenue for further research.

Nevertheless, certain models, such as *C. elegans*, may not fully capture the complexities of various human disorders due to limited genetic similarity [20] and dietary factors [21]. Additionally, differences in environmental factors and the complexities of maintenance render the use of zebrafish less optimal [17]. Conversely, while mammals like rats and mice exhibit the highest genetic homology with humans, considerations regarding research costs and time requirements must also be addressed [22]. Among these model organisms, *D. melanogaster* holds particular importance in nutrition and metabolism research due to its genetic similarities to humans and other mammals. Previous studies have tested the efficacy of curcumin in treating leaky gut using *D. melanogaster* [23] and have evaluated the pharmacological effects of curcumin in *D. melanogaster* from both phenotypic and molecular perspectives [24]. Its use in experiments provides valuable insights into the effects of nutritional interventions on biological processes relevant to human health, largely due to the conserved insulin growth factor (IGF) and mammalian target of rapamycin (mTOR) pathways shared with vertebrates, making it an effective model for studying growth disorders [25,26]. With approximately 75% genetic conservation with humans, *D. melanogaster* offers a valuable platform for investigating various phenomena [27]. Furthermore, utilizing *D. melanogaster* presents numerous advantages, including a short lifespan (2–3 months), ease of care, relatively low cost, exemption from ethical code requirements, and a genetic makeup nearly identical to that of humans and other mammalian species [25,28].

Building on this rationale, the aim of this study was to investigate the effects of undernutrition on both the early and later stages of *D. melanogaster*, with the goal of examining the phenotypic characteristics and molecular profiles associated with stunting-like phenotype. This comprehensive investigation encompasses various aspects of development, motor activity, and the long-term effects on lifespan, fertility, and the expression of genes related to metabolism, cell proliferation, and survival.

Methods

Experimental study design and groups

This experimental study employed wild-type *D. melanogaster* (Oregon R) strain, which was obtained from the Laboratory of Host Defense and Responses at Kanazawa University, Kanazawa,

Japan. Second-instar larvae were used in most experiments, except for gene expression analysis, which utilized third-instar larvae. All experiments were conducted under standard conditions (25°C, 12-hour light/dark cycle) in culture vials containing either normal or low-nutrient food. The schematic of the experimental design used in this study is presented in **Figure 1**. The study consisted of four groups where larvae were randomly assigned to each group to minimize bias. One control group received a standard nutrient-rich diet and three experimental groups in which larvae were subjected to low-nutrient diets (**Figure 1**). Low-nutrient diets were prepared by reducing the concentration of all ingredients (sucrose, glucose, yeast, and cornmeal) to 50%, 25%, and 12.5% of the standard levels, respectively. The emergence of a stunting-like phenotype was evaluated by assessing developmental growth, larval size and weight, locomotor activity, fecundity, the expression of development-related genes (*dilp5*, *srl*, *pepck*, *totA*, and *indy*), and the lifespan of adult flies emerging from the treated larvae (**Figure 1**). The developmental growth was determined by measuring the percentage of larvae transitioning to pupae and the percentage of pupae transitioning to adult flies. Determination of larval size, including weight, length, and width, was used as an indicator of stunting. Locomotor function was assessed using a negative geotaxis assay, and fecundity was determined by counting the percentage of offspring produced from mating between male and female flies in each group, which were fed either normal or nutrient-deficient food. Gene expression analysis was conducted using RT-qPCR, focusing on genes associated with metabolism, cell proliferation, and mitochondrial biogenesis. A survival assay was performed to examine the lifespan of adult flies that emerged from pupae resulting from mattings of adult flies treated with either normal or nutrient-deficient food.

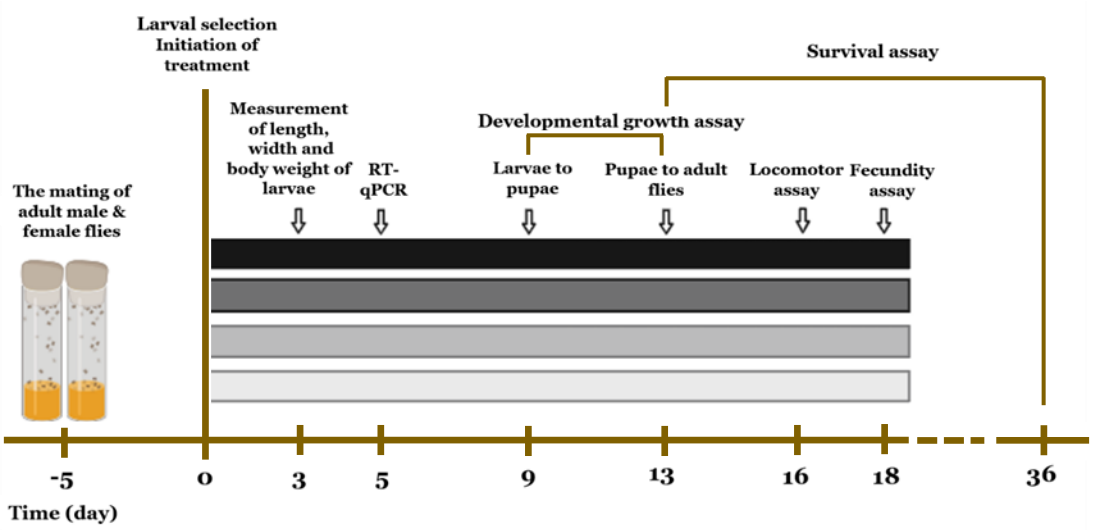

Figure 1. Experimental design used in this study. RT-qPCR, reverse transcriptase quantitative PCR

Preparation of *Drosophila melanogaster* food

A variety of diets consisting of normal food [29] and nutrient-restricted fly food were formulated (**Table 1**). In *Drosophila* diets, cornmeal, yeast, agar, and sugar serve critical roles: cornmeal and yeast supply carbohydrates and proteins essential for growth and metabolic energy, while agar maintains food structure and moisture, and sugar provides a primary energy source to support metabolic health and development [30,31]. Together, these components ensure proper nutrient availability, supporting larval growth, cell function, and developmental transitions. In states of undernutrition, such as stunting, protein levels are notably compromised. Insufficient protein prevents *Drosophila* larvae from synthesizing the cellular components required for normal growth. This protein deficiency adversely affects both cell proliferation and differentiation [32]. All ingredients, excluding methylparaben and propionic acid, were weighed, and water was added to achieve a 100-milliliter volume, followed by homogenization as recommended previously [33]. The mixture was heated on a hotplate at 100°C for 25 minutes and continuously stirred until slightly thickened. Subsequently, methylparaben and propionic acid were added, and the prepared food was transferred into vials to solidify further.

Table 1. *Drosophila melanogaster* food composition based on the contents of sucrose, glucose, yeast, and corn meal

Food materials	Normal fly food 100%	50% nutrient-deficient	25% nutrient-deficient	12.5% nutrient-deficient
Agar	1.5 g	1.5 g	1.5 g	1.5 g
Sucrose	3 g	1.5 g	0.75 g	0.375 g
Glucose	6 g	3 g	1.5 g	0.75 g
Yeast	1.25 g	0.625 g	0.3125 g	0.125 g
corn meal	5 g	2.5 g	1.25 g	0.625 g
Propionic acid	400 µL	400 µL	400 µL	400 µL
Methyl paraben	450 µL	450 µL	450 µL	450 µL
Aqua	ad 100 mL	ad 100 mL	ad 100 mL	ad 100 mL

Measurement of body weight, length and width of *Drosophila melanogaster* food

The objective of this assay was to investigate the impact of nutritional restriction on the changes in weight, length, and width of *D. melanogaster* larvae after being fed either normal or undernutrition food. Prior to weighing and measuring the larvae, the medium containing the second instar larvae was diluted with a 0.9% NaCl solution. The larvae's weight was measured using an analytical balance, with the second instar larvae placed on an object glass that had been previously weighed [34]. Length and width measurements are critical, as they represent different aspects of growth: length indicates longitudinal growth, while width reflects lateral changes. Both methods are essential for understanding the effects of undernutrition on organism morphology [35]. The measurement results were used to calculate the average body weight of the larvae. For length and width measurements, Image Raster Ver. 3.7 (Miconos) was employed. Ten-second instar larvae were selected from each group and individually examined under a stereo zoom microscope connected to a computer. Once the images of the larvae were displayed on the computer screen, their dimensions were systematically measured.

Developmental growth assay

The developmental growth assay was conducted to evaluate whether larvae can successfully progress to pupae and/or adult flies under conditions of nutritional deficiency. This approach enables researchers to understand the effects of nutrient deprivation on the developmental process, including its impact on growth progression and overall development of *Drosophila* [36]. This method entails monitoring and documenting each developmental transition, from larva to pupa and from pupa to adult fly. The success rate of the larva-to-pupa transformation and the emergence of adult flies from pupae are carefully observed and recorded. The results are expressed as the percentage of larvae successfully transforming into pupae and the percentage of pupae successfully emerging as adult flies.

Locomotor assay

The manifestation of low or impaired organismal responses to gravity was reflected in the negative geotaxis data, potentially indicating specific conditions or concerns within the flies [37]. In our experiment, a negative geotaxis assay was used. To conduct this experimental procedure, the vial was sterilized using a 70% alcohol solution. Empty vials were prepared for each treatment group (one vial per treatment group), and a circular line was drawn at the top of each vial, approximately 8 cm from the top edge, serving as the finish line. Then, 10 flies were transferred into locomotor treatment vials, which were categorized based on treatment type. Locomotor treatment vials were tapped three times onto the testing table to ensure the flies settled at the bottom of the vial.

Subsequently, the number of flies that crossed the finish line within 15 seconds was counted, and the percentage of negative geotaxis was calculated for each treatment group. This test was repeated three times [24,37]. The choice of 15 seconds as the cut-off time was based on a previous study that utilized a similar timeframe for assessing mobility and activity levels in *Drosophila* [38]. This duration provides a sufficient window to capture a broad range of locomotor abilities while minimizing the influence of fatigue that could affect the flies' performance. The number of flies that successfully passed the finish line was then counted.

Fecundity assay

A fecundity assay was carried out with the purpose of evaluating the reproductive capacity of *D. melanogaster*, specifically assessing its ability to produce viable offspring in response to nutritional variations [39]. In this assay, five pairs of male and female adult flies were mated in normal and treatment feed. After that, they were divided into groups of three vials and placed in fresh vials with normal (100%) and undernutrition food, which contained 50%, 25%, and 12.5% of the standard food concentration. After five days of mating, all of the flies were taken out of the vials. The total number of pupae and adult flies that mated in each group were counted and observed daily [40].

Survival assay

A survival assay was conducted to investigate the impact of dietary variations on the lifespan and survival rates of adult flies. This assay provides insights into how nutrient deficiencies influence overall health and longevity [41]. In this experiment, three vials containing ten Oregon-R larvae each were utilized to represent both normal food and treatment food. The larvae were transferred to vials containing either normal food or food with reduced nutritional value and maintained at 25°C. The food in each vial group was changed every three days to ensure food quality [42,43]. Feeding was conducted from the larval stage through to adulthood. The survivorship of the flies was monitored daily until all flies in the experimental group had died [44].

Gene expression analysis

This method was employed to explore the expression profiles of specific genes that have been reported to play a significant role in nutritional regulation in *Drosophila*, namely *dilp5*, *srl*, *pepck*, *totA*, and *indy*. For total RNA isolation, live larvae were used. Specifically, ten third instar *D. melanogaster* larvae were individually collected and placed into tubes. Total RNA was then isolated using the PureLink RNA Mini Kit (Invitrogen, Thermo Fisher Scientific, Carlsbad, USA), following the manufacturer's instructions. The total RNA content of each sample was quantified using a spectrophotometer (BioDrop, Biochrom, Cambridge, UK).

The quantification of targeted gene expression levels was conducted through reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) in accordance with the manufacturer's instructions. Each RT-qPCR reaction was carried out in a volume of 10 µL using SuperScript III Platinum SYBR Green One-Step RT-qPCR with ROX (Invitrogen, Waltham, USA). Using target gene-specific primers (**Table 2**), RT-qPCR was conducted using the protocol involved an initial cycle at 37°C for 15 mins, followed by subsequent cycles at 95°C for 10 mins, and 40 cycles at 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 30 seconds.

A standard melting curve analysis was incorporated into the RT-qPCR run to validate the specificity of the anticipated product amplification. Additionally, the RNA levels of the target gene were assessed using a set of rp49 primers, as detailed in **Table 2**, with *rp49* serving as an internal reference to normalize the expression levels. Following processing with Rotor GeneQ (Qiagen, Hilden, Germany), gene expression analysis was performed on all of the resultant data.

Table 2. List of RT-qPCR primers used in this study

Genes	Forward Primers	Reverse Primers
dilp5	5'-GCCTTGATGGACATGCTGA-3'	5'-CATAATCGAATAGGCCCAAGG-3’
srl	5'-CTCTTGGAGTCCGAGATCCGCAA-3	5'-GGGACCGCGAGCTGATGGTT-3'
pepck	5'-CCGCCGAGAACCTTATTGTG-3'	5'-AGAATCAACATGTGCTCGGC-3'
indy	5'-CTGCCCAACTCTGTCCTCTTACTG-3'	5'-CAGGATCAGGTACAGAGGATGGAT-3’
totA	5'-CCCTGAGGAACGGGAGAGTA-3'	5'-CTTTCCAACGATCCTCGCCT-3'
rp49	5'-GACGCTTCAAGGGACAGTATCTG-3'	5'-AAACGCGGTTCTGCATGAG-3’

Data analysis

All data were processed utilizing GraphPad Prism 9, with a minimum of three independent biological replicates. Survival data analysis was conducted employing the log-rank test (Kaplan-Meier method). For the examination of negative geotaxis, a two-way ANOVA (followed by the post hoc Tukey) was implemented. One-way ANOVA followed by the post hoc Dunnett analyses were employed in the evaluation of development, reproduction, and gene expression. The results

of these statistical analyses were visually represented via bar graphs. Data presentation included mean values accompanied by standard deviation (mean±SD), and statistical significance was determined at $p<0.05$.

Results

Impairment of growth and development in *Drosophila melanogaster* due to undernutrition

In this study, we investigated the progression of larval growth and development into pupae and adult flies in response to undernutrition. Our data revealed significant differences in the transition of larvae to pupae (**Figure 2A**) and from pupae to adult flies (**Figure 2B**), with a dose-dependent decrease in the percentage of larvae developing into pupae and adults as nutrient concentration decreased. However, no statistically significant differences were observed between larvae receiving 50% of the standard food content and those fed with normal fly food. In contrast, larvae maintained on nutrient-deficient diets with 25% and 12.5% food concentrations were unable to normally develop into pupae and adult flies.

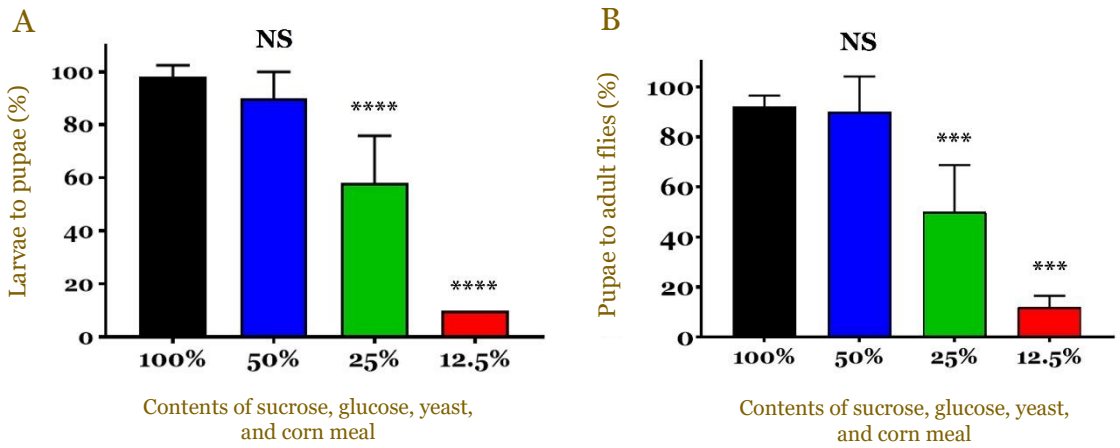

Figure 2. Developmental stages of *Drosophila melanogaster* from larvae to pupae (A) and from pupae to adult flies (B). Significant differences were observed between the normal food (100%) and the treated diets, which contained sucrose, glucose, yeast, and cornmeal at concentrations of 25% and 12.5%, over nine days for larvae to pupae and 13 days for pupae to adult flies. Compared to the normal food conditions, the percentage of *D. melanogaster* larvae developed into pupae and adult flies was significantly affected by diets with reduced nutrient content. NS: non-significant; significant at *** $p<0.001$ and **** $p<0.0001$.

Undernutrition can affect the growth and physiological development of organisms, leading to impaired development. In this study, measurements of the weight (**Figure 3A**), length (**Figure 3B**), and width (**Figure 3C**) of *D. melanogaster* larvae supported this observation. We found that larvae fed nutrient-deficient diets exhibited lower weights and reduced body sizes compared to those receiving a standard nutrient-rich diet.

Early death phenotype in *Drosophila melanogaster* induced by undernutrition

A survival assay was conducted to evaluate the influence of nutrient availability on the lifespan of *D. melanogaster*. The objective of this investigation was to observe significant alterations in the survival rates of the flies (**Figure 4**). In this study, the log-rank test was used to statistically compare survival curves between two or more groups subjected to different experimental treatments. Survival testing was conducted after all pupae had emerged into adult flies, starting on day 13 post-treatment.

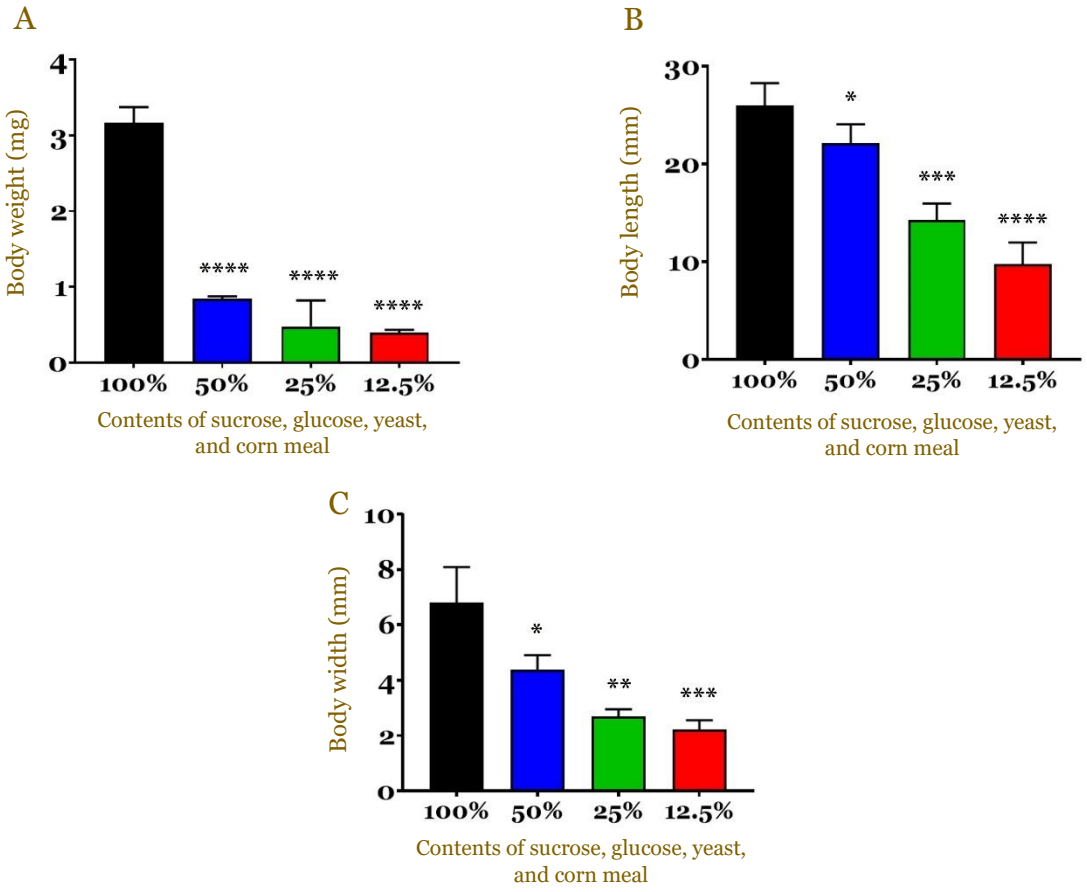

Figure 3. Comparison of the weight (A), length (B), and width (C) of *Drosophila melanogaster* between normal food and undernutrition food, indicating that the physiological body size of living organisms can be impeded by diminished nutrient content over a three-day period. The reduction of the weight, length, and width of *D. melanogaster* larvae was observed for each undernutrition food group in comparison to the normal food group. Significant at * $p<0.05$; ** $p<0.01$; *** $p<0.001$ and **** $p<0.0001$.

The results further demonstrate a proportional decrease in the lifespan of *D. melanogaster* with the nutrient level of the food; the lower the nutrient content, the shorter the lifespan of the flies. The food with 12.5% nutrient content exhibited the most rapid decline in survival on the second day, followed by a gradual reduction over 12 days for the food with 25% nutrient content. The food with 50% nutrient content exhibited a decline in survival over 36 days, whereas flies fed normal food displayed a complete decrease in survival over 38 days (**Figure 4**).

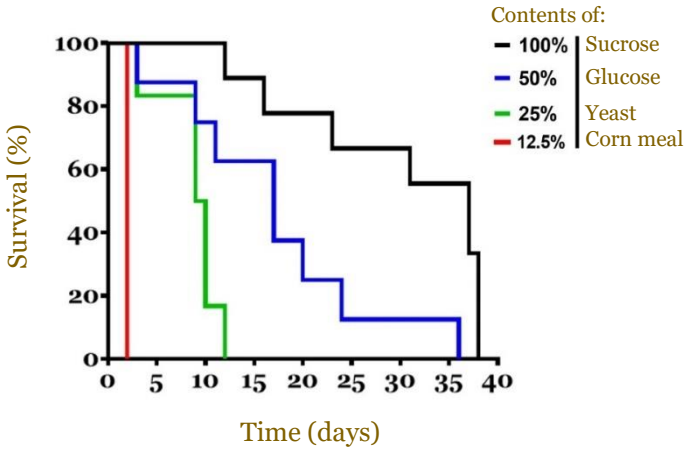

Figure 4. Significant differences in survival rates of adult *Drosophila melanogaster*, with a greater ability to survive observed in those on a normal food diet compared to those on the treatment food with lower nutrient content.

Insignificant changes in the locomotor of *Drosophila melanogaster*

In addition to the survival assay, a locomotor assay was conducted to evaluate the impact of feeding on motor abilities and response to the gravity of *D. melanogaster* (**Figure 5**). This approach provided insights into the phenotypic consequences of nutritional deficiencies. Notably, the absence of specific nutrients did not influence the motor function of *D. melanogaster*. It is deduced that this result has implications for the life expectancy of the treatment group with the lowest nutrient content of 25% on day 15 and 12.5%, as they did not endure long enough for subsequent days of locomotor testing to occur.

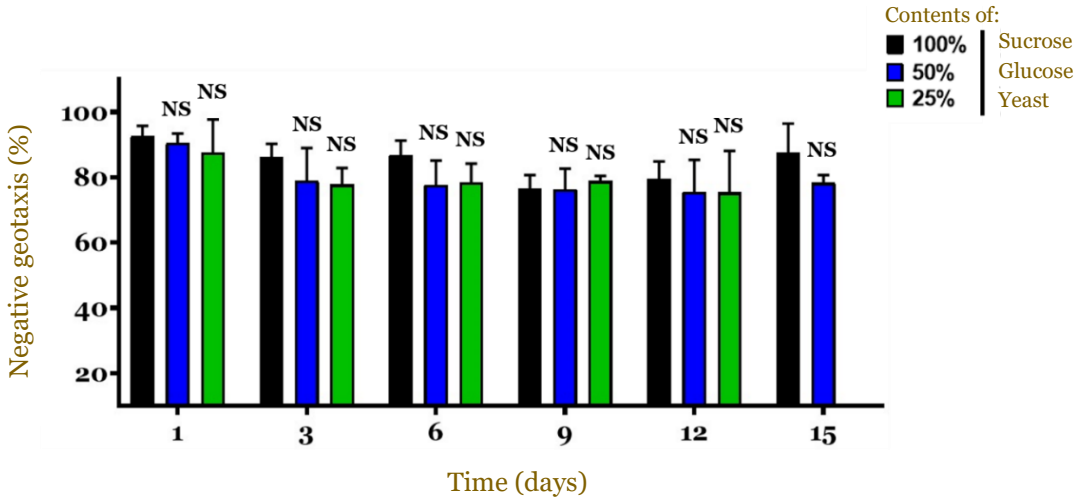

Figure 5. No significant alterations in the locomotor of *Drosophila melanogaster*. The locomotor activity remained non-significantly consistent across all concentrations during the experiment. NS: non-significant.

Impairment of *Drosophila melanogaster* fecundity due to undernutrition

This analysis was conducted to identify the impact of nutrition on the reproductive development and offspring production of *D. melanogaster*. Our data indicated that the intake of nutrients could impact the number of offspring produced by *Drosophila* (**Figure 6)**. Consistent with observations in developmental stages, a decrease in nutrient content in the treatment food was associated with a lower number of offspring produced by *Drosophila*.

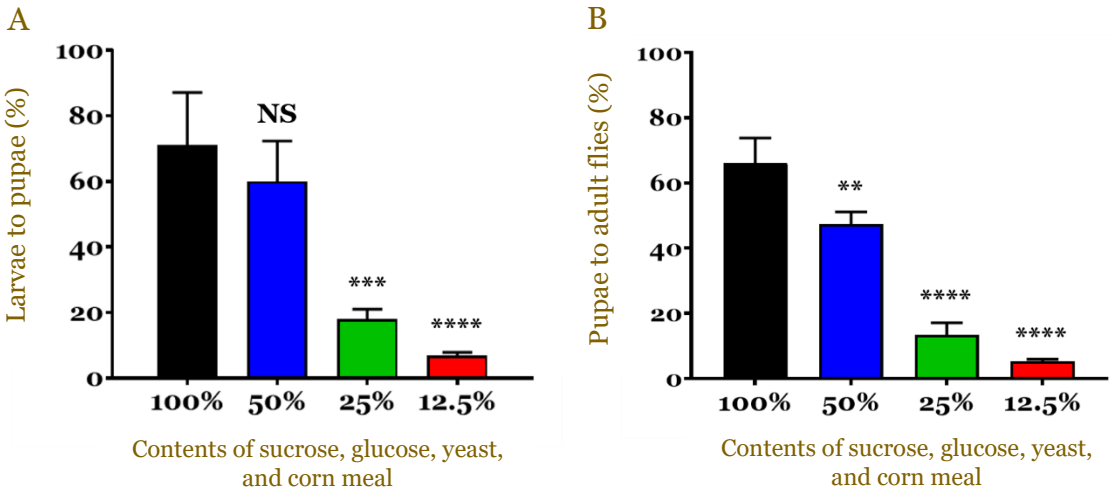

Figure 6. The fecundity of *D. melanogaster* is adversely affected by the nutritional content of the food, as indicated by the development from larvae to pupae (A) and from pupae to adult flies (B). NS: non-significant; significant at $^{**}p<0.01$; significant at $^{***}p<0.001$ and significant at $^{****}p<0.0001$.

Modulation of expression of metabolic genes by undernutrition

To complement the phenotypic findings, a molecular analysis of gene expression was performed. Larvae fed with low-nutrient diets exhibited a notable increase in *dilp5* expression (**Figure 7A**). This increase is associated with elevated levels of Unpaired 2 (encoded by *upd2*), a cytokine that promotes the release of *dilp5* from insulin-producing cells (IPCs), which are responsible for synthesizing and secreting insulin. A similar trend was observed for the *srl* gene, which also showed increased expression under nutrient-deficient conditions (**Figure 7B**). However, when larvae were fed diets containing 50% of the normal concentrations of sucrose, glucose, yeast, and cornmeal, the changes in gene expression were not statistically significant.

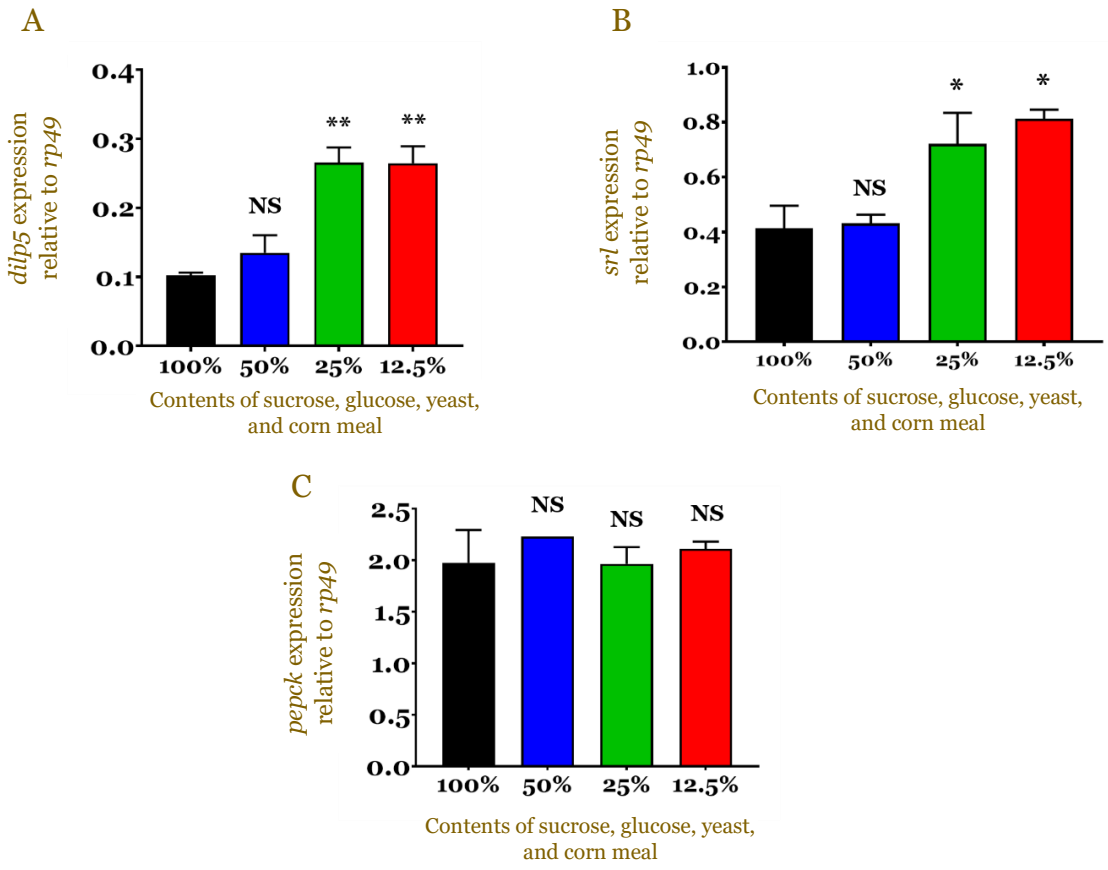

Figure 7. A significant increase in the expression of the *dilp5* (A) and the *srl* (B), with an insignificant change in the expression of the *pepck* (C), due to undernutrition. NS: non-significant; significant at $^{*}p<0.05$; significant at $^{**}p<0.01$.

Furthermore, an examination of the *pepck* gene, responsible for encoding the *Phosphoenolpyruvate Carboxykinase* (*pepck*) involved in gluconeogenesis, serves as the principal regulator of glucose homeostasis. This analysis aimed to demonstrate that *pepck* gene can enhance gluconeogenesis, a process linked to nutrition that boosts glucose production during nutrient deficiency. Upon comparison with the control group consuming normal food, no statistically significant differences were observed across all treatment groups, as illustrated in the data chart (**Figure 7C**), which included food with 50%, 25%, and 12.5% less sucrose, glucose, yeast, and corn meal.

Downregulation of Jak/STAT-related gene due to undernutrition

The result showed that the expression of the *totA* gene was downregulated as the nutrient content in the food was reduced (**Figure 8**). While no significant change in *totA* expression was observed in larvae fed with 50%-deficient food, a significant downregulation was noted in larvae fed with 25% and 12.5% nutrient-deficient food. This downregulation of *totA* expression correlated with

impaired larval growth and development, as evidenced by delayed progression through developmental stages and reduced emergence of adult flies (**Figure 8**).

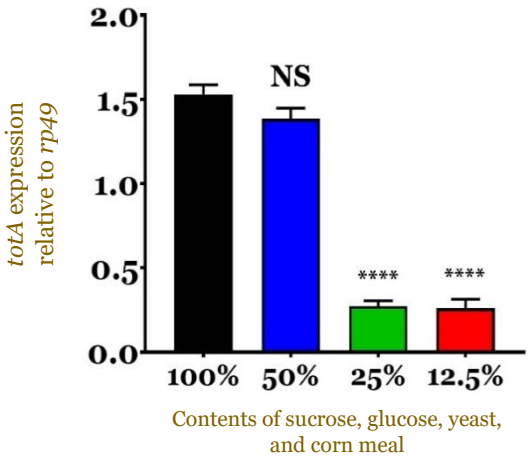

Figure 8. Downregulated expression of *totA* due to undernutrition. NS: non-significant; significant at $^{****}p<0.0001$.

Increased expression of genes involved in mitochondrial biogenesis due to undernutrition

To assess the impact of diet on aging-related genes during the lifespan of flies, it is crucial to identify genes beyond those solely linked to aging that also influence growth. The *indy* gene encodes a transporter protein that regulates citrate uptake in cells, serving as a key intermediary in cellular metabolism. In both *Drosophila* and mammals, the *indy* gene has been associated with various metabolic processes, including mitochondrial biogenesis. The "I'm not dead yet" (*indy*) gene, analogous to the human *SLC13A2* gene, is among the genes recognized for triggering mitochondrial biogenesis and influencing the aging process [45]. The result showed that the expression of *indy* is increased in a manner dependent on the reduction of nutrition in the food (**Figure 9**).

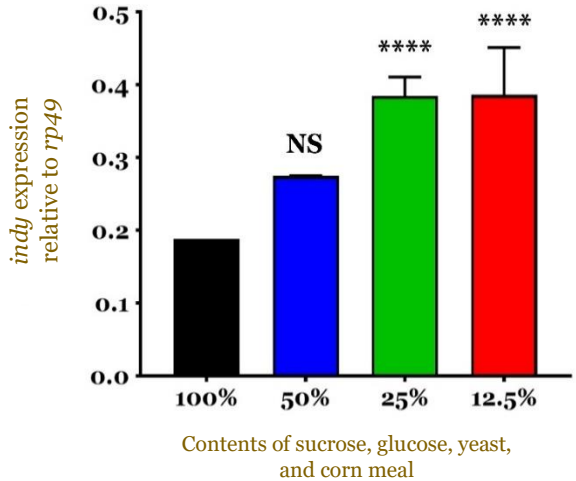

Figure 9. The augmented expression of *indy* by undernutrition. NS: non-significant; significant at $^{****}p<0.0001$.

Discussion

Our observations indicate that undernutrition significantly affects *D. melanogaster*, resulting in growth retardation, reduced larval body size, impaired fecundity, and decreased survival rates. Indeed, deficiencies in protein and micronutrients have been shown to reduce growth quality in *Drosophila*, highlighting the adverse effects of undernutrition on health and development

[46,47]. In addition to developmental assessments, this study incorporated survival analysis to examine the lifespan of the flies and assess the influence of nutrient intake on their survival. The survival assay revealed that flies fed nutrient-deficient food had a shorter lifespan compared to those fed a normal diet, suggesting that reduced nutrient content contributes to decreased fly longevity. These findings align with studies on malnourished rats, where inflammation was shown to reduce survival, paralleling the results observed in this study [9].

Changes in the phenotypic features observed in *D. melanogaster* upon treatment with nutrient-deficient food may be associated with alterations in the expression of genes related to metabolism, cell proliferation, and survival. To investigate this, molecular analysis was performed alongside phenotypic assessment to evaluate the impact of nutrient availability on *D. melanogaster* development. The expression levels of genes such as *dilp5, srl, pepck, totA*, and *indy* were examined due to their relevance to metabolic processes and development. Transcriptional analysis was conducted using RT-qPCR to assess gene expression. In this study, metabolism-related genes, including *dilp5, srl,* and *pepck*, were evaluated to determine their involvement under nutrient-deficient conditions. Previous research has explored the role of these genes in regulating physiological traits such as lifespan, development, reproduction, and dietary restriction, as well as the balance between carbohydrate storage, circulation, and metabolic regulation [48]. *Drosophila* insulin-like peptides (Dilps), including Dilp5 (encoded by *dilp5*), play a crucial role in regulating glycogen and fat storage by inhibiting gluconeogenesis and promoting glycolysis in cells [49,50]. In nutrient-deficient larvae, *dilp5* expression increases as survival rates decline, while under normal nutritional conditions, *dilp5* expression decreases, leading to improved survival. These findings align with a study that reported a two-fold reduction in *dilp5* mRNA levels, which coincided with an extension of lifespan [48].

Reduced food intake impairs the ability of insulin producing cells (IPCs) to release Dilp into the bloodstream, leading to accumulation of Dilp within the IPCs and reduced levels in the hemolymph. A similar phenomenon is observed in humans, where nutrient deficiency diminishes the response of IGF, a hormone essential for regulating growth and metabolism. During feeding, *Drosophila* body fat releases Upd2, a cytokine that regulates the secretion of dilp5 from IPCs [51]. When *Drosophila* were fed a high-sugar diet compared to a control diet, the relative expression of *dilp2*, *dilp3*, and *dilp5* was observed to be twice as high [50]. This elevation is associated with increased expression of the *srl* gene, which results in higher Spargel protein levels in *Drosophila*, suggesting improved mitochondrial function and enhanced cellular respiration, leading to greater energy production [52].

In this study, the expression of the *srl* gene increased as the nutritional content of the food decreased (**Figure 7B**). Given that PGC-1 is critical for energy metabolism and mitochondrial function, these findings suggest the activation of pathways involved in these processes. However, no statistically significant differences in *srl* expression were observed across the treatment groups compared to the control group consuming normal food (**Figure 7C**). Additionally, the increase in *pepck* gene expression is likely a result of dietary restrictions due to low nutritional or carbohydrate intake [53]. This shortage of glucose triggers the organism's gluconeogenesis pathway, which is initiated by the Pepck enzyme, to generate glucose and fulfill energy requirements. Overall, the results show that undernutrition disrupts both molecular mechanisms and phenotypic traits in organisms, including *D. melanogaster*. This study provides valuable insights into the effects of nutrient deficiencies on growth and development, contributing to a better understanding of undernutrition and its potential impact on long-term health outcomes.

The findings of this study demonstrate that nutrient deficiencies can impair organism growth and development (**Figure 10**). Exploring drug compounds that target the long-term effects of stunting using the *D. melanogaster* model is essential. Future research should include measurements of body parameters such as body weight, body length, and body width in adult flies exhibiting a stunting-like phenotype. These measurements will provide valuable data for further investigations and offer a framework for studying potential solutions for treating stunting. The findings hold promise as potential targets for therapeutic interventions aimed at addressing undernutrition.

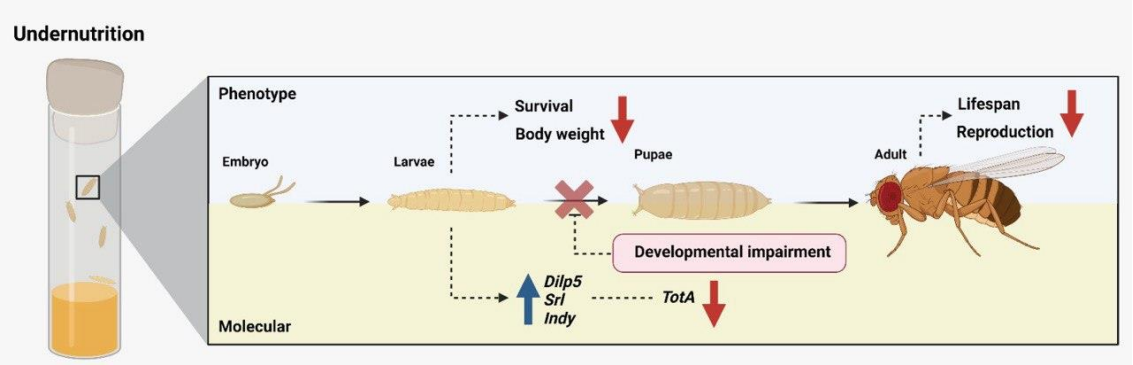

Figure 10. Undernutrition-induced retarded growth accompanied by multiple phenotypical and molecular changes in *Drosophila melanogaster*.

Undernutrition observed in vivo using *D. melanogaster* may reflect similar effects in humans, where protein deficiency impairs physical growth and metabolic development. We acknowledge several limitations in our study, including the lack of data on adult *Drosophila* phenotypes and a focus on protein deficiency, while other nutritional factors that could significantly influence growth and development were not explored. Despite these limitations, this research emphasizes the need for further studies on nutrition-related topics. Addressing these knowledge gaps could improve our understanding of the complex relationship between nutrition and metabolic health, guiding future dietary recommendations for both humans and model organisms. Such investigations will undoubtedly contribute to advancing our knowledge in the field of nutrition. Given these effects, we propose that *D. melanogaster* is a promising model organism for simulating undernutrition and establishing stunting-like phenotypes. This potential utility may be particularly valuable for future research efforts focused on exploring pharmacological targets related to the long-term effects of stunting.

Conclusion

This study highlights the significant impact of nutrient deficiencies on growth, development, and reproductive capacity in *D. melanogaster*. The observed stunting-like phenotypes and altered molecular expressions demonstrate that *D. melanogaster* is a promising model for studying the effects of undernutrition and stunting. Our findings reveal that nutrient-restricted diets result in reduced larval growth, impaired survival, and decreased fertility while not affecting locomotor activity. Molecular analysis further identified key gene expression changes, including upregulation of *dilp5*, *srl*, and *indy*, and downregulation of *totA*, providing insights into the mechanisms underlying the organism's response to nutrient stress. These results contribute to a better understanding of the molecular pathways involved in undernutrition and offer potential targets for future research on stunting-related conditions.

Ethics approval

Not required.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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