# THE IMPACT OF EARLY LIFE MALNUTRITION ON FEEDING BEHAVIOR IN *Drosophila melanogaster*

A THESIS SUBMITTED TO

**THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY**

THE UNIVERSITY OF TRANS-DISCIPLINARY
HEALTH SCIENCES & TECHNOLOGY

FOR THE PARTIAL FULFILMENT OF THE AWARD OF THE

DEGREE OF
M.Sc. LIFE SCIENCES (AYURVEDA BIOLOGY)

BY
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Under the Guidance of

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NATIONAL CENTER FOR CELL SCIENCES, PUNE

December 2022- June 2023

**THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY**

**Private University Established in Karnataka by ACT 35 of 2013**

**BENGALURU - 560064**

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## DECLARATION BY THE CANDIDATE

I declare that this thesis "**THE IMPACT OF EARLY LIFE MALNUTRITION ON FEEDING BEHAVIOR IN *Drosophila melanogaste*r**" submitted for the award of Master of Science to THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY, Bengaluru, is my original work, conducted under the supervision of Dr. Gaurav Das ( and co-supervision of, Dr. Megha). I confirm that no part of the work reported herein has been submitted for a degree or examination at any other university. References, funding and material obtained from other sources have been duly acknowledged, and no part of this dissertation has been plagiarised.

**Place: NCCS Pune**

**Signature of the Candidate**

**Date: 07-07-2023**

**Name of candidate: Asmita Dogra**

**Reg. No.: 2021MScAB02**

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## CERTIFICATE FROM THESIS SUPERVISOR/S

This is to certify that the work incorporated in this thesis **“THE IMPACT OF EARLY LIFE MALNUTRITION ON FEEDING BEHAVIOR IN *Drosophila melanogaste*r”** submitted by Asmita Dogra was carried out under my/our supervision. No part of this thesis has been submitted for a degree or examination at any other university. References, help and material obtained from other sources have been duly acknowledged. I confirm the originality of the work and that there is no plagiarism in any part of the thesis.

| Name, Designation | Role | Signature, Date |
|---|---|---|
| Dr. Gaurav Das | Supervisor | Gaurav Das |

# Acknowledgement

I would like to express my heartfelt gratitude and appreciation to the individuals who have made significant contributions to the successful completion of my M.Sc. dissertation. Their guidance, support, and expertise have been invaluable throughout this research journey.

First and foremost, I would like to extend my deepest thanks to my dissertation guide, Dr. Gaurav Das. His profound knowledge, unwavering support, and exceptional mentorship have been instrumental in shaping the direction and quality of this research work. His valuable insights, constructive feedback, and constant motivation have pushed me to explore new perspectives and strive for excellence. I am truly grateful for his dedication, patience, and guidance throughout the entire dissertation process.

I would also like to express my sincere thank to my co-guide, Dr. Megha. Her expertise in the field, insightful suggestions, and collaborative approach has significantly enhanced the depth and breadth of this research. I am deeply grateful for her contributions and encouragement throughout this journey.

I extend my sincere thanks to my PhD mentor Miss Bhavna Pydah who willingly shared her time and knowledge for this study. I would also like to thank Miss Radhika and all the members of Brain and Feeding behavior lab, NCCS for their valuable contributions and cooperation that have been instrumental in generating meaningful findings and insights.

I would also like to thank Mr. Debashish from Stunted fly lab for teaching me the basic fundamentals of fly husbandry.

I would also like to express my gratitude to my friends and colleagues for their support, encouragement, and stimulating discussions. Their camaraderie and shared experiences have provided a sense of community and motivation during challenging times.

Lastly, I would like to extend my deepest appreciation to my family for their unconditional love, encouragement, and understanding throughout my academic journey. Their constant support, sacrifices, and belief in my abilities have been the foundation of my success. I am forever grateful for their patience, understanding, and the strength they have provided me

# SUMMARY

Early-life malnourishment has been demonstrated to impact the growth and development of various species, including humans, resulting in both phenotypic and metabolic changes that can adversely affect an organism's quality of life and render them more vulnerable to feeding-related disorders. Therefore, it is crucial to comprehend the influence of early-life malnourishment on an organism's feeding behavior. In our investigation, we examined the consequences of early-life starvation on feeding behavior. We induced early-life starvation in flies by limiting the diet of third instar larvae to a 100 mM sucrose agar. We observed that these flies consumed less food compared to control flies; however, their food intake appeared to be regulated by their body weight, as there was no alteration in consumption when normalized by body weight. Through olfactory-associated conditioning, we discovered that the starved flies exhibited reduced memory reinforced by 500 mM sucrose. Furthermore, we observed that these early-life starved flies displayed increased susceptibility to reduced sensitivity to sweet taste when reared on high glucose concentrations. Based on our preliminary findings, we speculate that early-life starved flies may be more prone to exhibiting altered behavior when exposed to unconventional diets. Nevertheless, further experiments are required to elucidate the molecular and neural mechanisms underlying the modified feeding behavior induced by early-life starvation.

# PERSONAL REFLECTION

Undertaking my master's dissertation project has been a profound and transformative journey, leaving me with a multitude of personal reflections on the skills gained, challenges overcome, and the invaluable knowledge acquired along the way. Personally, I feel that my science communication skills have significantly improved through engaging in journal club meetings. These sessions provided me with the opportunity to present and discuss research articles, honing my ability to effectively convey complex scientific concepts to my peers. Additionally, working in the laboratory environment has taught me invaluable lessons in adaptability and collaboration. I quickly learned the importance of fitting in with the team, navigating different personalities and communication styles, and fostering a positive and cooperative work atmosphere. Moreover, I gained a deeper understanding that acquiring data is only the initial step; the true essence lies in the interpretation and analysis of the results. This realization has guided my approach in designing subsequent experiments based on the insights gleaned from previous findings. In terms of technical skills, I had the privilege of learning how to standardize protocols through collaboration with my knowledgeable PhD seniors. Their guidance allowed me to refine my experimental procedures and ensure consistency in my results. Furthermore, at the beginning of my thesis, I had the opportunity to delve into the world of confocal imaging with the assistance of a PhD senior. On a personal level, working in a new laboratory environment was an amazing experience. Overall, my master's dissertation project has not only expanded my scientific knowledge but has also nurtured personal growth and resilience in the face of challenges, preparing me for future endeavors in my academic and professional journey.

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# INTRODUCTION

## Background

Malnutrition encompasses deficiencies, excesses, or imbalances in an individual's consumption of energy and/or nutrients. This term encompasses two primary categories of conditions. The first category is "under-nutrition," which includes stunting (insufficient height for age), wasting (inadequate weight for height), underweight (insufficient weight for age), and micronutrient deficiencies or insufficiencies (insufficient essential vitamins and minerals (*The UNICEF/WHO/WB Joint Child Malnutrition Estimates (JME) Group Released New Data*, n.d.). The second category comprises overweight, obesity, and diet-related non communicable diseases, such as heart disease, stroke, diabetes, and cancer .The prevalence of under-nutrition and malnutrition is notably high in underdeveloped and developing countries.

Malnutrition affects individuals in all countries, as 144.0 million children under 5 suffer from stunting, 47.0 million children under 5 were wasted of which 14.3 million were severely wasted, and 38.3 million were overweight, globally (*The UNICEF/WHO/WB Joint Child Malnutrition Estimates (JME) Group Released New Data*, n.d.).

In the year 2020, an estimated 149 million children under the age of 5 experienced stunted growth globally. Specifically in India, around 46.6 million children were observed to have stunted growth, as reported in the Global Nutrition Report of 2018. Undernutrition accounts for 50% of mortality in children under the age of 5 in India (*Malnutrition*, n.d.). These findings provide the foundation for our investigation into the impact of early life malnutrition.

## Malnutrition: effects and consequences

Malnutrition can arise from inadequate consumption or excessive intake of calories, protein, carbohydrates, vitamins, or minerals (Astley & Finglas, 2016). Insufficient nutrition during pregnancy or infancy can lead to permanent impairments in physical and cognitive development

(Prado & Dewey, 2014) (Benton, 2010), (Astley & Finglas, 2016). Starvation and severe undernourishment are associated with diminished stature, muscle wasting, low energy levels, and edema (Shetty, 2006). However, even seemingly minor symptoms of malnutrition, such as increased infection rates and fatigue, can occur. Over-nutrition, often coupled with a sedentary lifestyle, is another form of malnutrition that heightens the risk of disability and diseases like cancer and cardiovascular disorders, thereby reducing quality of life, productivity, and lifespan (Friedrich, 2015). Factors contributing to malnutrition encompass poverty, socioeconomic status, agricultural productivity, and food security, influenced by various elements like global warming, conflict, and disease outbreaks (Arthur et al., 2015). Protein-energy malnutrition (PEM) or severe acute malnutrition (SAM) represents a specific form of malnutrition characterized by acute deficiencies in caloric intake, macronutrients, and sometimes micronutrients (Ngo et al., 2016), (Galler et al., 2021) .

Malnutrition can stem from internal factors like eating disorders (e.g., anorexia nervosa, bulimia nervosa) or external factors like famine. Longitudinal studies have indicated that mothers with a history of anorexia nervosa or bulimia nervosa may give birth to infants with reduced head circumference and delayed neuro-cognitive development (Marcos, 2000) . These effects may not only be attributed to under-nutrition but also maternal stress during pregnancy.

Malnutrition not only detrimentally affects cognitive abilities but also impacts motor skills, social abilities, and mental health, with some of these effects persisting into adolescence and adulthood (Champakam et al., 1968); (Galler et al., 1983), (Galler, 1984), (Upadhyay et al., 1989), (Berkman et al., 2002), (Roger et al., 2022) . Plausible links have been suggested between prenatal under-nutrition and neuro-developmental outcomes, including poorer cognitive function, language delay, and increased risk of psychiatric diseases in offspring . These studies strengthen the evidence that early-life malnutrition exerts negative effects on neural development and contributes to neuropsychiatric disorders (Roger et al., 2022).

Malnutrition is classified as a pathological condition resulting from inadequate dietary intake, impaired nutrient absorption, and heightened energy expenditure (Saunders & Smith, 2010). It leads to dysfunction across multiple organ systems, including cardiac atrophy in malnourished individuals. Prolonged malnutrition induces alterations in gastrointestinal (GI) function, pancreatic exocrine function, intestinal permeability, and nutrient absorption (Saunders & Smith, 2010). Additionally, it disrupts the immune system by compromising cell-mediated immunity, increasing susceptibility to infections, and delaying wound healing (Saunders & Smith, 2010) (Green, 1999), (Stratton et al., 2003). Extensive research has demonstrated the profound impact of malnutrition on neurodevelopment, ultimately influencing an individual's behavioral patterns.

Maternal malnutrition during gestation and lactation is recognized to exert detrimental effects on offspring (Papathakis et al., 2016). It is hypothesized that achieving stability during early developmental stages could influence long-term health outcomes. This notion raises the significance of investigating the impact of early life malnutrition on growth, development and behavior.

## Early life malnutrition and its effects

Nutrition plays a vital role in the overall growth and development of organisms. Optimal nutrition during early life stages is particularly crucial for proper organism development (FrankovÁ & Barnes, 1968), (Winick, 1975). The primary function of food is to provide the necessary nutrients to meet the body's energy and metabolic requirements, ensuring adequate growth and development. Food also has the ability to regulate specific physiological functions, promoting health and reducing the risk of disease.

However, early life malnutrition can have significant impacts on the development of an individual's organ systems, leading to impaired functioning across multiple systems. Research indicates that inadequate fetal nutrition, such as protein restriction, can alter the allocation of energy to various systems during development, with a focus on protecting the brain at the expense of other organs (Hales & Barker, 2001). This can result in changes in kidney, pancreas, and liver function, as well as hormonal and sympathetic nervous system regulation, and adipose tissue (Antonow-Schlorke et al., 2011).

Early life malnutrition poses a risk factor for conditions such as type 2 diabetes, liver diseases, long-term effects on the interaction between microbes and the host, immune system dysfunction, neurological issues, neuro-developmental disorders, and behavioral problems (Barker et al., 1993) . It is well-established that nutritional deficiencies in early life can have enduring adverse effects on learning and behavior.

Numerous studies have demonstrated that early-life malnutrition leads to structural and functional alterations in the brain (Dobbing, 1970) (Dobbing & Sands, 1973) (Winick, 1975). The impact of nutritional deficiencies during early life on behavior and cognitive development has been extensively investigated. School-age children who experienced malnutrition in early childhood generally exhibit lower IQ levels, impaired cognitive function, reduced academic achievement, and increased behavioral problems compared to matched controls and, to a lesser extent, siblings (Grantham-McGregor, 1995). In mammals, including humans, severe prenatal malnutrition has negative effects on neural development and complex behaviors such as sleep, memory, and learning (Olivares et al., 2023).

Animal-based experiments have the potential to reveal the relationships among early-life malnutrition, alterations in brain structures, and the possible behavioral and cognitive problems (Kahsai & Zars, 2011). Due to the extremely high similarity to human beings in general brain development, rodents have been used to understand the process of brain development in early life. Many animal studies exhibit that malnutrition in early life may affect the morphology, neurochemistry, and neurophysiology of the hippocampal formation, the main brain region associated with spatial learning and memory (Castro & Rudy, 1989), (Prado & Dewey, 2014). Some studies also demonstrate that prenatal or neonatal nutritional deficiency may epigenetically reprogram some gene expression patterns related to adult behavior, learning and memory (Zúñiga-Hernández et al., 2023).

## Early life malnutrition affects animal growth and behavior

A wealth of scientific research has provided compelling evidence regarding the adverse effects of early-life malnutrition on animal growth and behavior.

Among various behavioral aspects, learning has received particular attention as an area affected by early malnutrition (Kallen, 1973). An animal's performance in learning situations is influenced by multiple factors, including motivation level, reinforcement value, and emotional state. It has been observed that early experience with food restriction can lead to differences in adult feeding behavior (Levitsky & Barnes, 1973). In rats, these effects manifest as increased food hoarding, elevated consumption rate, and heightened instrumental responding for food

(Massaro et al., 1974). Similarly, dogs subjected to early food restriction also exhibit an increased rate of eating (Manteca, 2011).

In avian studies, early-life malnutrition has been associated with impaired growth, specifically marked by reduced femur bone length in malnourished cockerels. Even when provided with an adequate diet later in life, these individuals fail to attain the same size as the control group (Cravioto & Robles, 1965). Likewise, pigs exposed to early-life malnutrition exhibit increased excitability in response to aversive stimuli and experience overall size reduction. During the depletion period, protein or calorie-deprived pigs display a gaunt and unthrifty appearance. Protein-depleted animals show diminished food intake, while calorie-depleted pigs retain their appetite. Importantly, behavioral abnormalities resulting from a short period of early-life malnutrition persist even after complete nutritional rehabilitation and months following the initial nutritional insult. Furthermore, impaired learning ability has been observed in these malnourished pigs (Barnes et al., 1970).

Studies involving rats have demonstrated that pre-weaning food deprivation leads to more significant growth retardation and behavioral abnormalities in response to early-life malnutrition, particularly in male individuals. Memory impairment has been documented in rats experiencing combined pre- and post-weaning food deprivation compared to control groups. Additionally, early deprived rats exhibit slightly smaller size compared to normal controls. Early deprivation also leads to impaired learning behavior, although the specific contribution of altered motivation versus altered capacity remains unclear (Barnes et al., 1966). Another rat study revealed that malnourished pups had lower average body weights compared to controls and exhibited impaired performance in various behavioral tests (FrankovÁ & Barnes, 1968).

Malnutrition, including starvation, represents a common and significant stressor resulting from environmental and seasonal fluctuations in food availability. In fish, malnutrition can induce reductions in body mass, energy reserves, and metabolic function, thereby impacting swimming ability. For instance, continuous malnutrition during larval development in zebrafish has been linked to decreased larval survival and body growth from 5 to 14 days post-fertilization. Swimming performance, encompassing measures such as distance, velocity, motility, maximum duration of movement, and mobile states, significantly diminishes under malnutrition conditions (Fan et al., 2021).

Furthermore, reinforcement value as it relates to food in malnourished animals has been investigated. For example, a study involving weaned rats maintained on an extremely low protein diet until sixty days of age. Subsequently, these rats were tested in a multiple y-maze and rewarded with either a balanced diet or a protein-deficient diet upon traversing the maze. The study revealed that the incentive value of reinforcement differs for animals maintained on different diets, leading to impaired learning scores (Griffiths & Senter, 1954).

Regarding Drosophila, it is shown that larvae exposed to a low-yeast diet resulted in smaller adult size (Tu & Tatar, 2003). Additionally, drosophila subjected to nutritional stress during the late larval stages exhibited impaired growth, characterized by diminished body length and weight compared to the control group. These malnourished flies also demonstrated distinct metabolic and behavioral phenotypes in comparison to the control group, including impaired climbing behaviors and altered metabolic characteristics (Patil et al., 2022).

In conclusion, numerous studies reported in the scientific literature have consistently indicated detrimental effects of early

## ***Drosophila melanogaster*** **as a model organism**

*Drosophila melanogaster*, commonly known as the fruit fly, shares numerous physiological, metabolic, nervous system and genomic similarities with humans, indicating evolutionary conservation. Approximately 50% of the fruit fly's genes show homology with genes found in mammals, suggesting a common ancestry. Moreover, around 75% of genes associated with human diseases have orthologs, genes with similar functions, in the fruit fly.

Drosophila melanogaster possesses several advantageous characteristics that make it an excellent model organism. Various genetic tools enable easy manipulation of neurons, and ethical concerns associated with mammalian models are absent when working with fruit flies. The short life cycle of fruit flies is a major advantage, as a large number of flies can be generated within a short timeframe. From egg fertilization to the emergence of an embryo takes only 24 hours, and after three larval stages, it matures into an adult fly in just 10 days (Hannah Simmons & Deepthi Sathyajith, 2018).

A female fruit fly can produce up to 1500 eggs during her lifetime, providing a continuous supply of new individuals for genetic studies. The small size and minimal requirements of Drosophila make it feasible to raise and conduct experiments with a large number of flies in a small laboratory setting, without the need for extensive resources (Hannah Simmons & Deepthi Sathyajith, 2018).

Genetically, Drosophila offers several advantages as a model organism. It has only four pairs of chromosomes compared to the 23 pairs in humans. This simplicity facilitated the mapping of genes and investigation of genetic inheritance in early Drosophila genetic studies. The entire genome of Drosophila has been sequenced and annotated, similar to the human genome, although the fly's genome significantly smaller, constitutes only 5% of its size (Hannah Simmons & Deepthi Sathyajith, 2018).

Drosophila possesses distinct anatomical features, such as wings and eyes, which allow for easy characterization. These genetic markers can be readily identified and studied under a microscope. Additionally, Drosophila exhibits behaviors similar to those observed in humans, including eating, mating, and sleeping. Therefore, studying the impact of genetics on human behavior can be assessed using this model organism (Hannah Simmons & Deepthi Sathyajith, 2018).

In Drosophila melanogaster, the brain undergoes development through the emergence of approximately one hundred neuroblasts during the initial round of neurogenesis at embryonic stage 9. These neuroblasts divide and give rise to ganglion mother cells (GMCs) and additional neuroblasts until the larval stage begins. Subsequent divisions of neuroblasts result in the production of two neurons each, contributing to the formation of the primary lineage and the generation of neurons. After a temporary pause in primary neuroblast expansion, the second lineage emerges and continues from the larval stage to the late pupal stage. During this period, the organism relies on nutrients obtained from the environment rather than yolk resources (Spindler & Hartenstein, 2010). As a result, the nutritional conditions experienced during the second expansion stage have the potential to influence the epigenetic configuration and lineage of neuroblasts, subsequently impacting synapse formation and neuronal excitability, which can give rise to behavioral disorders (Hillman & Chen, 1981), (Dauncey & Bicknell, 1999), (Xu et al., 2014). Drosophila melanogaster serves as an appropriate model organism for studying epigenetic and transcriptional changes, even in specific subpopulations of neurons, in response to early-life nutritional scarcity and their potential correlation with alterations in behavior (Aristizabal et al., 2020).

The brain of Drosophila consists of mushroom bodies, a conserved brain structure found in insects. The mushroom bodies comprise an adult neuropile that contains approximately 2000 Kenyon cells (KCs) per lobule (Shih et al., 2019). The KCs can be divided into three main neuronal types, namely α/β, α′/β, and γ. The development of the mushroom bodies initiates during the second phase of neuroblast expansion in the larval stage, with γ KCs differentiating before the third instar larval stage, and the α/β and α′/β KCs differentiating during the late third instar and pupal stages. The mushroom bodies are involved in associative learning and complex behaviors such as walking, feeding, olfactory memory, and sleep (Zúñiga-Hernández et al., 2023).

## Rationale of the study

Our objective is to investigate the impact of malnourishment during early life on the feeding behavior of adult Drosophila melanogaster. Previous research has indicated that flies subjected to nutritional stress during the late larval stage experience metabolic alterations that may affect their behavior (Patil et al., 2022). Hence, our goal is to examine whether early-life starvation in a Drosophila model leads to any deficits in memory and learning abilities.

# MATERIALS AND METHODS

## Fly Lines

All the flies were reared at 25°C and 60% humidity on food containing sugar, corn flour, malt extract, and yeast granules, with a 12 hr/12 hr light and dark cycle in incubators. Fly strain used for all the experiments is CS-BZ.

All ELS flies during their third larval stage were reared on 100mM sucrose and 2% agar medium.

## Chemicals and other materials

Sucrose (ANJ Biomedicals 57-50-1), Dextrose (ANJ Biomedicals 50-99-7), Type 1 agar (HIMEDIA GRM666-500G), Triglyceride reagent (Sigma Aldrich T2449), Glycerol standard solution (Sigma Aldrich G7793), Free glycerol reagent (Sigma Aldrich F6428), Bradford's reagent (Sigma Aldrich B6916), PBS Tablets (MP Biomedicals 2810305), Tween- 20 (MP Biomedicals Q779R), Whattman filter paper (GE 3030-917), Octan-3-ol (Sigma Aldrich 218405), 4-Methyl cyclohexanol (Sigma Aldrich 153095), Propionic acid, Methylparaben.

## Generation of ELS flies

To generate ELS adult flies, regular CS-BZ flies were allowed to lay eggs on the laboratory diet. Subsequently, the eggs were allowed to undergo a maturation period of 90 hours, during which they developed into third-instar larvae (L3 stage). Milli-Q water was added into the fly food bottle containing L3 larvae, followed by pouring of the larvae onto a petri plate. Subsequently, a total of 95-100 third-instar larvae were carefully collected using a brush and transferred to either the standard laboratory diet (water stressed control flies) or sucrose agar diet (ELS flies). After eclosion, adult flies were aged on a lab diet.

## Measurement of fly weight

5 days old flies were anesthetized using ice and subsequently sorted into males and females. Each group consisting of 25 flies was individually weighed using a precise balance inside a microfuge tube. The weighing process was repeated for consecutive days over a period of seven days while the flies were maintained in DIETS vials.

## DIETS assay for feeding measurements

In order to quantify the food consumption of flies, we prepared 1% agar vials with a volume of 8 ml each. We used micro-centrifuge tube caps as food cups and poured laboratory diet onto the food cups using a fixed pipette with a volume of 200 ul. The food cups were then attached to the agar vials. Male and female flies, aged three days, were added separately to the vials and allowed to acclimate for 48 hours. The food cups and agar vials were replaced every 24 hours during this period. Throughout the experiment, the DIETS vials were kept horizontally.

On the third day, fresh food cups were prepared and their initial weight was measured. The food cups were then attached to the agar vials, and the flies were transferred to the fresh DIETS vial. After 24 hrs, the flies were transferred to new DIETS vials, and their initial weights were measured. The final weight of the food cups was also recorded. To account for evaporation control, some DIETS vials were kept without flies, and their weights were measured as well.

This process was repeated for a total of seven days.

## Triacylglycerides quantification

Preparation of the TAG reagents:

PBST: PBS ( 1 tablet in 100 ml Mili-Q water) + 0.05% Tween 20.

Standard preparation: dilute 40 ul of the glycerol standard solution with 60 ul of PBST i.e. 1mg/ml triolein equivalent solution. Two-fold serial dilution is done (50 ul of 1 mg/ml + 50 ul PBST) to generate 0.5,0.25,0.125mg/ml standards.

Working TAG reagent: mix triglyceride reagent and free glycerol reagent in a 1:4 ratio (as per TAG assay kit instructions).

To measure the levels of triglycerides in the whole bodies of the flies, we used 5-day-old flies that were fed a laboratory diet. The flies were anesthetized on ice, and five flies were collected per micro-centrifuge tube, separating males and females. The flies were weighed after collection.

To extract the triglycerides, 500 ul of PBST (Phosphate-Buffered Saline with Tween-20) was added to each micro-centrifuge tube, and the samples were homogenized using a mechanical homogenizer. The micro-centrifuge tubes were then centrifuged at 4000 g and 4°C for 15 minutes. The supernatant was carefully transferred to new micro-centrifuge tubes.

To measure the protein content of the samples, 10 ul of the supernatant was taken and mixed with Bradford's reagent. The Bradford assay relies on the color change of the reagent in the presence of protein. Bovine Serum Albumin (BSA) was used as a standard, and milli-Q water served as a blank. After adding 300 ul of Bradford's reagent to each well, the samples were incubated at 37°C for 30 minutes. The absorbance at 595 nm was then measured using a spectrophotometer to quantify the protein content.

For the triglyceride measurement, the remaining supernatant was heated at 70°C for 10 minutes. In a 96-well plate, 20 ul of glycerol standard solutions were added as references, using PBST as a blank, and 20 ul of the samples were added to subsequent wells. Next, 200 ul of a working TAG (triglyceride) reagent was added to each well, and the plate was incubated at 37°C for 10 minutes. The absorbance at 540 nm was measured using an ELISA plate reader to quantify the levels of triglycerides.

## Proboscis Extension Reflex (PER) suppression assay: Tarsal PER

After emerging from their pupal stage, the flies were matured on a laboratory diet for a period of seven days. On the eighth day old, the flies were transferred to different media containing corn flour, yeast, and varying concentrations of glucose (5%, 10%, and 20%). They were allowed to feed on these diets for three days. Subsequently, on the eleventh day, PER suppression assay was performed on flies starved for 24 hours on 0.75% agar.

When the flies had reached 23 hours starvation they were stuck on a glass slide using nail polish. Subsequently, they were allowed to recover in a humidified chamber for 30 min. Their Proboscis extension reflex (PER) was measured by tarsal stimulation with 5%, 10% and 20% glucose.

Firstly, flies that had been grown on a diet consisting of seven days of laboratory diet followed by three days of a 5% glucose diet were used. Water was presented to the flies, followed by the presentation of a 5% glucose solution to their tarsi using a pipette. The extension of the proboscis was observed under a microscope. This process was repeated three times with the 5% glucose solution. Afterwards, water was again presented to the flies to remove any remaining traces of the 5% glucose solution. The same procedure was then carried out with 10% and 20% glucose solutions.

The aforementioned steps were then repeated with flies that had been grown on a diet consisting of seven days of laboratory diet followed by three days of a 10% glucose diet, as well as flies that had been grown on a diet consisting of seven days of laboratory diet followed by three days of a 20% glucose diet.

## Sucrose acuity test using T-maze setup

### Setup

The ability of flies to detect and prefer varying concentrations of sucrose was assayed in a T-maze setup. The T-maze consists of an elevator which brings the flies to the choice point where flies are allowed to choose between sucrose-agar or agar paper lined tubes attached to the T-maze and placed opposite to each other at a 180 degree angle.  Agar or sucrose agar papers were prepared by pouring a thin uniform layer of molten (sucrose) agar solution on the whatman filter paper measuring 7.5 cm by 4.8 cm. These papers were rolled and placed inside acrylic tubes such that they line the inner walls of the tubes. The tubes are then attached to the T-maze.

### Experiment

5-6 day old flies were starved for 24 h on 0.75 % agar. They were gently tapped into the T-maze elevator and allowed to recover for 30 sec. Subsequently, the elevator was lowered and the flies were given 2 min to make a choice between agar and sucrose agar. After 2 min the flies were trapped in their respective tubes. Subsequently, they were collected and the number of flies in each arm was counted. Acuity index was measured using the formula below.

Acuity index = (#No. of flies choosing sucrose-agar - #No. of flies choosing agar)/ Total No. of flies that made choice

## Learning and memory test using Y-maze setup

### Odor preparation:

For the preparation of odors used in Y-maze training and testing, two specific odors, Octanol and MCH, were employed. Initially, 2000 mg of mineral oil was carefully weighed on a precise balance and placed in a 5 ml micro-centrifuge tube. A stock solution of MCH was created by adding 12 ul of MCH to the mineral oil, and a stock solution of octanol was prepared by adding 4 ul of octanol to the mineral oil. To obtain working solutions, 625 ul of the MCH stock was mixed with 625 ul of mineral oil for MCH, while 312.5 ul of the octanol stock was mixed with 937.5 ul of mineral oil for octanol. Fresh preparation of the odors was carried out approximately 5-10 minutes prior to each experiment.

### Setting up training vials:

In order to set up the training vials, 0.75% agar vials and 0.75% + 500mM/200mM sucrose vials, each with a volume of 5 ml, were prepared for appetitive training. Small pieces of Whatman filter paper measuring 1 cm × 1 cm were inserted inside 3D printed odor cups. Subsequently, 20 ul of the working odors were spotted onto the filter papers placed within the odor caps. These odor cups are sieved hence, facilitated the diffusion of the odors into the vials and were sealed with tape. Empty vials with caps were utilized to house the flies during a 5-minute interval between exposure to the two odors.

**Appetitive olfactory conditioning (training):**

100 flies of 5-6 days old were starved on 0.75% agar at a temperature of 25°C. Prior to training, additional flies were added to the training vials for 20 minutes to induce the presence of fly odor as well as odor from the odor cups. Half of the fly groups were trained with Octanol as the conditioned stimulus ($CS^+$), while the other half were trained with MCH as the $CS^+$ in order to counteract any odor bias. The starved flies were first transferred to 0.75% agar vials to experience the $CS^-$ odor for 5 minutes as the initial conditioning phase. Subsequently, the flies were transferred to empty vials for 5 minutes before being exposed to the $CS^+$ odor vials containing sugar-agar for 5 minutes. Following the training session, the flies were once again kept on 0.75% agar vials for 24 hours of starvation.

**Setting up Y-mazes:**

Y-mazes were then set up for memory testing. 3D printed odor choice vials were employed, and Whatman filter paper pieces measuring 2.5 cm × 2 cm were spotted with 20 ul of either $CS^-$ or $CS^+$ odors. These filter papers were placed at the bottom of the vials, which were then attached to the two arms of the Y-maze connector. A fly loading vial was connected to the third arm of the Y-maze connector. The entire Y-maze setup was allowed to be odorized for 20 minutes.

**Memory testing using Y-maze:**

Memory testing using the Y-mazes involved loading the trained flies into the loading vial of the Y-mazes. Subsequently, the Y-mazes were placed upright in a plastic stand within a temperature-controlled environment (25°C). The negative geotaxis behavior of the flies ensured their climbing up towards either one of the odor vials, where they became trapped. A testing duration of 30 minutes was done. After freezing, the flies were counted in each vial, including those that did not make a choice. Each group trained with a specific odor was considered as a single experimental trial (n=1).

# RESULTS AND DISCUSSION

## Generation of early life starved adults

*Drosophila melanogaster* as a model to study early life starvation has been established by (Patil et al., 2022) . Flies subjected to 36 hours of nutritional stress during the late larval stage showed manifestation of phenotypic changes in the adult stage. Mainly these phenotypes showed significant reduction in weight and height in adults. It also resulted in metabolic changes in the ELS flies. They observed higher tag levels in hemolymph of male flies however no significant change was observed in the whole body tag for both males and females.

We wanted to replicate the ELS phenotype in CS-BZ flies to study the impact of early life malnutrition in feeding behavior. The protocol used to generate the ELS flies were followed as mentioned in (Patil et al., 2022). Briefly L3 larvae (90 hours old) were collected and transferred to either 100 mM sucrose agar media or normal diet and allowed to develop into adult stage (Fig 1A). Post eclosion adult flies were transferred to a normal lab diet. We observed ~40% reduction in the weight of ELS males (Fig 1B) and ~ 27% reduction in ELS females weight (Fig 1C). Although the weight of the flies is reduced we observed that weight did not revert back to as of controls when fed on a normal lab diet for seven days (Fig 1D).

In the previous report mentioned above higher TAG levels were observed only in the circulating hemolymph of ELS males however we observed that the total body tag levels for both males and females in ELS flies was higher than the control flies (Fig 1E).

Thus we were able to successfully generate CS-BZ strain ELS flies however the point to note is that there may be some difference in their metabolic profile.

# Figure 1

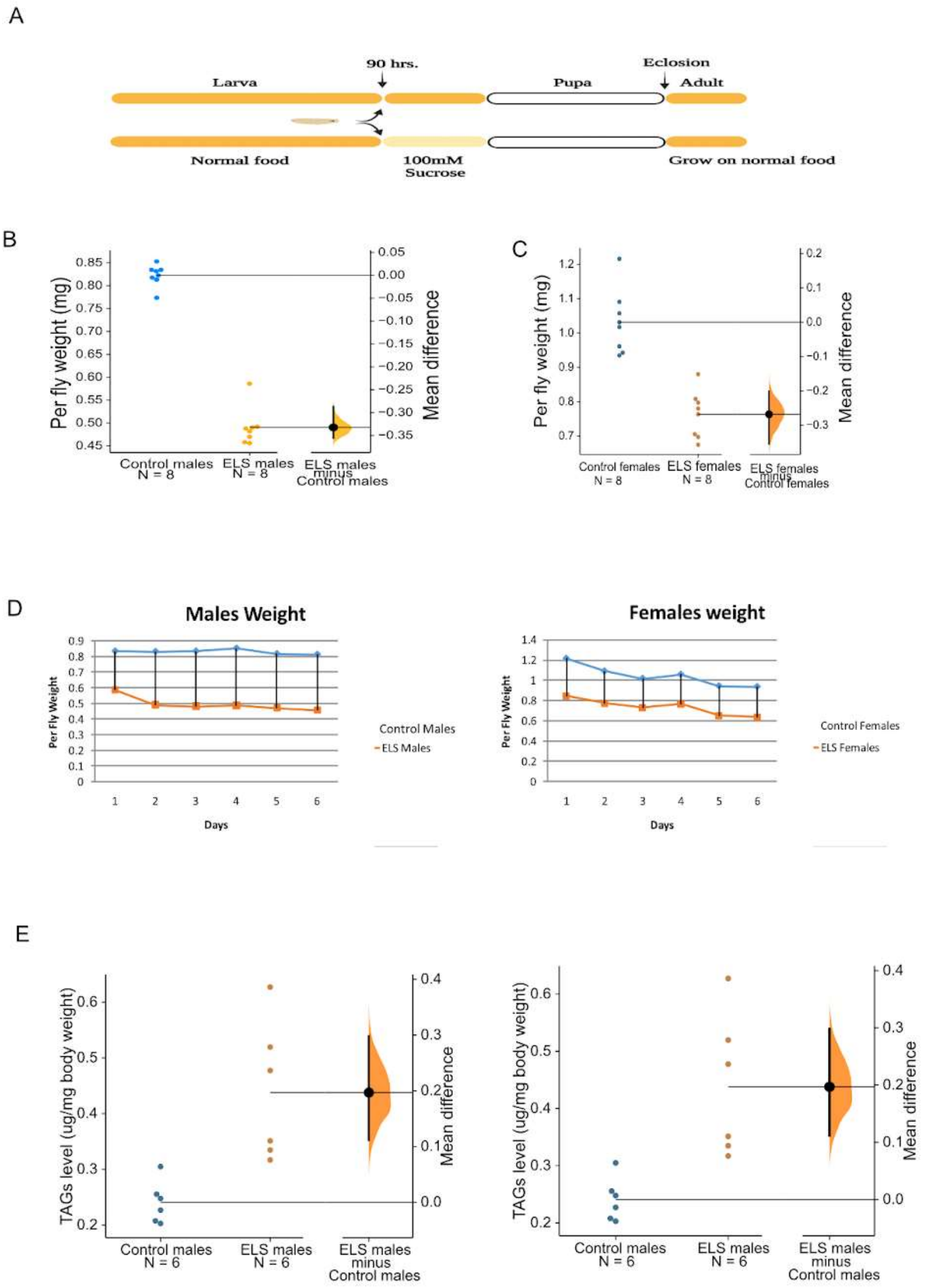

A
90 hrs.
Larva
Pupa
Eclosion
Adult
Normal food
100mM Sucrose
Grow on normal food
B
Per fly weight (mg)
Mean difference
Control males N = 8
ELS males N = 8
ELS males minus Control males
C
Per fly weight (mg)
Mean difference
Control females N = 8
ELS females N = 8
ELS females minus Control females
D
Males Weight
Per Fly Weight
Days
Control Males
ELS Males
Females weight
Per Fly Weight
Days
Control Females
ELS Females
E
TAGs level (ug/mg body weight)
Mean difference
Control males N = 6
ELS males N = 6
ELS males minus Control males
TAGs level (ug/mg body weight)
Mean difference
Control males N = 6
ELS males N = 6
ELS males minus Control males

Figure 1- Early life starved (ELS) flies have reduced weight and high body TAG levels.

A. Weight of flies after being on a normal diet for 4 days was measured. ELS males (~40%) and female flies (~28%) have reduced weight when compared to control flies.

B. The weight of 3 day old control and ELS flies was measured everyday for the next 7 days while being on a normal lab diet. The weight of ELS flies does not revert back to normal levels when flies are fed with normal diet during the adult stage.

C. In 3 day old flies, the whole body TAG normalized to the body weight of flies was measured. Elevated whole body TAG levels were observed in both ELS males and females.

Statistics for Figure A and C was done using estimation stats. The dark dot represents the difference between the means of 2 groups. The plot shows distribution of the mean difference after performing 5000 bootstrap resampling of the data. The dark line represents 95% CI of the distribution.

## Body weight dependent food consumption in ELS flies

We wanted to study whether early life malnutrition leads to the changes in the amount of food consumption in the adult stage. We measured the amount of normal lab food consumed by the flies using DIETS assay setup standardized in the brain and feeding behavior lab (ref). In this setup flies are allowed to consume food from a single food cup placed inside an agar vial and the amount of food consumed was calculated by the difference between the initial weight of cup (before feeding) and final weight of cup (after feeding) (Fig 2A). For more details of assay please refer to material and methods.

We observed that ELS males and females had a tendency to consume less amount of food over a seven day period of time when compared to control males and females flies respectively (Fig 2B). However when normalized by per fly weight, the amount of food consumed by control and ELS flies were comparable.(Fig 2C).

We assessed the effect of early life malnourishment on mortality caused due to starvation in the adult stage. For this 3 day old adult ELS and control flies were transferred to 0.75% agar only medium and the percentage survival was noted. We observed no difference in starvation resistance between control and ELS flies of both sexes ( Fig 2D)

This shows that flies that have experienced early life malnutrition consume less food compared to control flies. We speculate that the amount of food consumed by ELS flies is being controlled by their body weight. And hence these flies could sustain similar starvation stress as that of the control flies.

## Figure 2

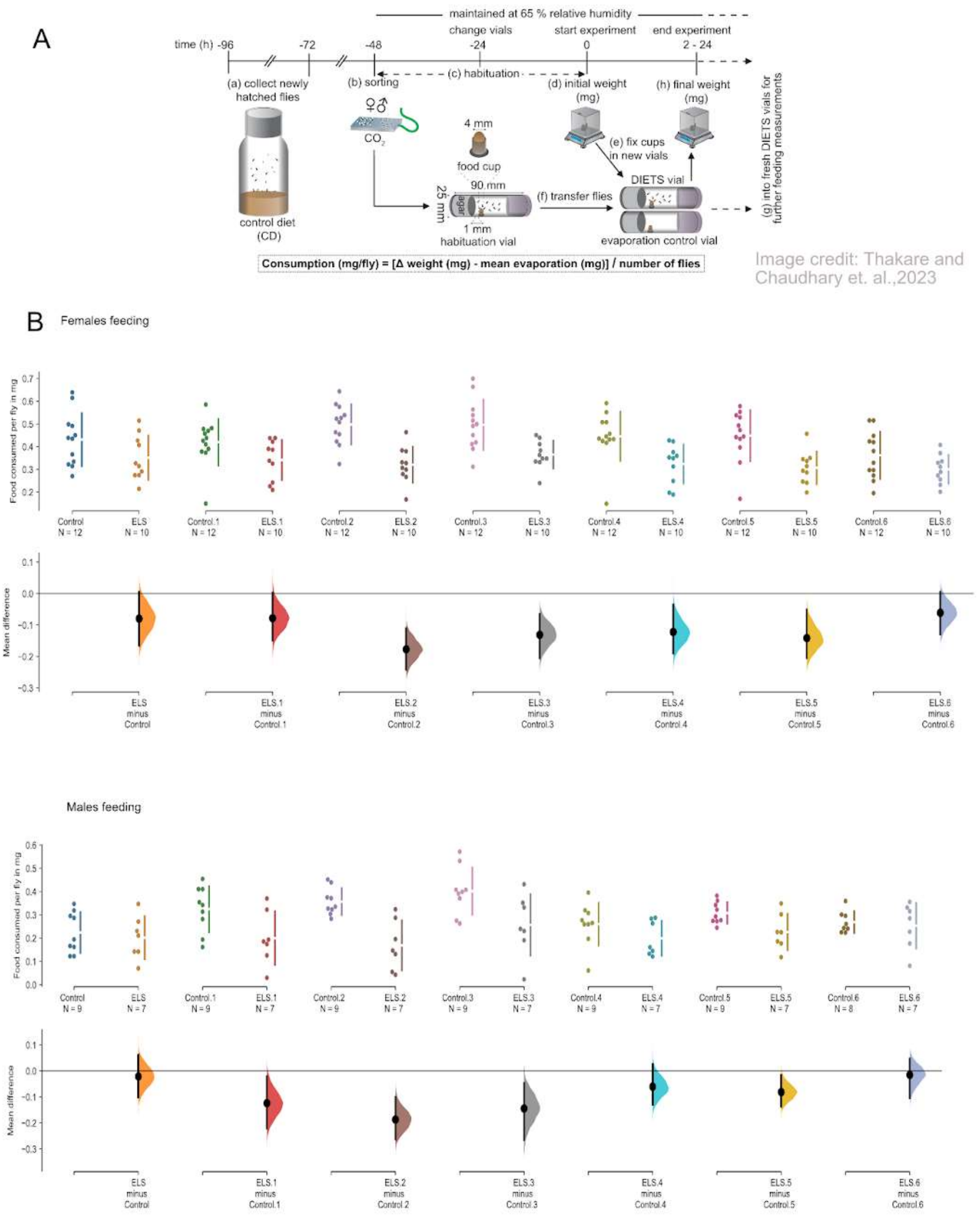
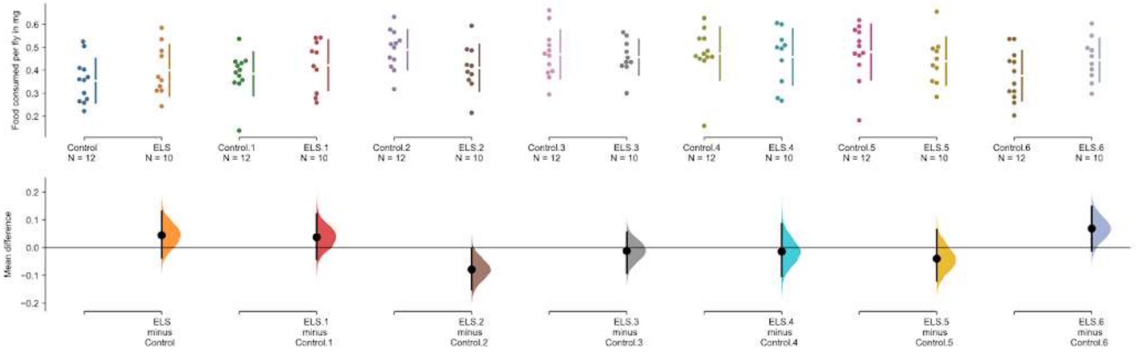


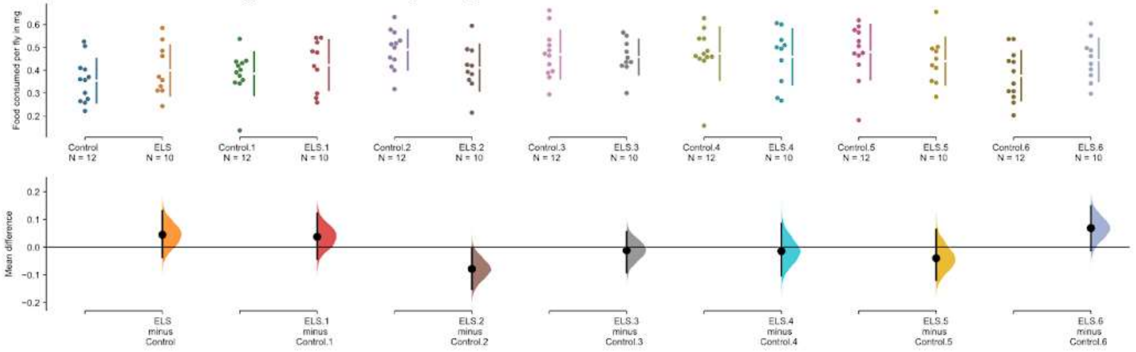
Image credit: Thakare and Chaudhary et. al.,2023

# Figure 2

C Females feeding normalized by weight



Males feeding normalized by weight



D

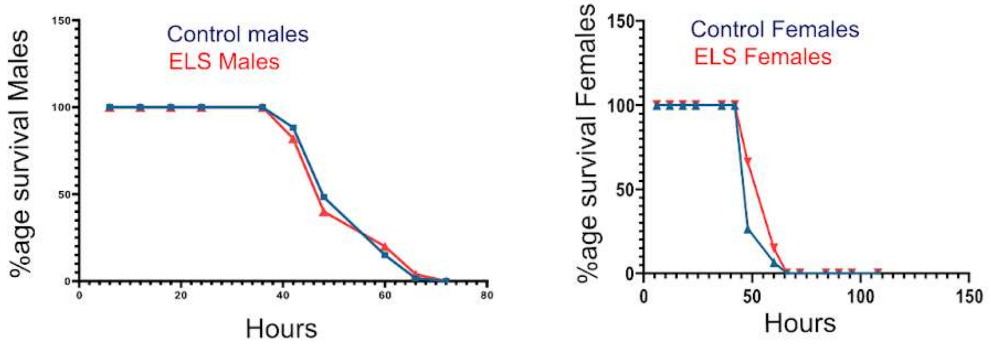


Figure 2-

A. Schematic representation of the DIETS assay performed to measure the amount of food consumed by ELS and control flies. In short, 3 day old flies were CO2 anesthetized and sorted into males and females. They were then habituated in DIET vials for 2 days while they recovered from anesthesia. Subsequently, they were transferred to DIET vials and the amount of food consumed/ day was measured for 7 days.

B. The amount of food consumed by ELS males and females was consistently less than the control flies throughout the 7 days.

C. Upon normalization with body weight, we observe that ELS and control flies show similar feeding over a 7 day period.

D. Starvation resistance of ELS males and females is comparable to that of the control flies

Statistics for Figure B and C were done using estimation stats. The dark dot represents the difference between the means of 2 groups. The plot shows distribution of the mean difference after performing 5000 bootstrap resampling of the data. The dark line represents 95% CI of the distribution.

**Mild suppression in sucrose reinforced memory in ELS flies**

We assayed odor associated sucrose reinforced memory in ELS flies. We subjected the flies to olfactory conditioning paradigm where the flies were first exposed to an odor in the presence of agar (no reward) known as conditioned stimulus negative, CS-. The flies were then transferred to an empty vial and subsequently to varying concentrations of sucrose agar vial in the presence of another odor known as conditioned stimulus positive, CS+ (Fig 3A).

ELS flies did not show any complete disruption in sucrose reinforced memory. However, mild decrease in 500 mM sucrose reinforced memory was observed in ELS flies compared to control flies (Fig 3B). Interestingly, 200 mM sucrose reinforced memory in ELS flies was comparable to that of control flies (Fig 3C).

We speculate that since ELS flies have alteration in their metabolism, they may be unable to differentiate robustly between post-ingestive reward from different concentrations of sucrose.

However our data is preliminary and requires more experiments which could validate our hypothesis.

Figure 3

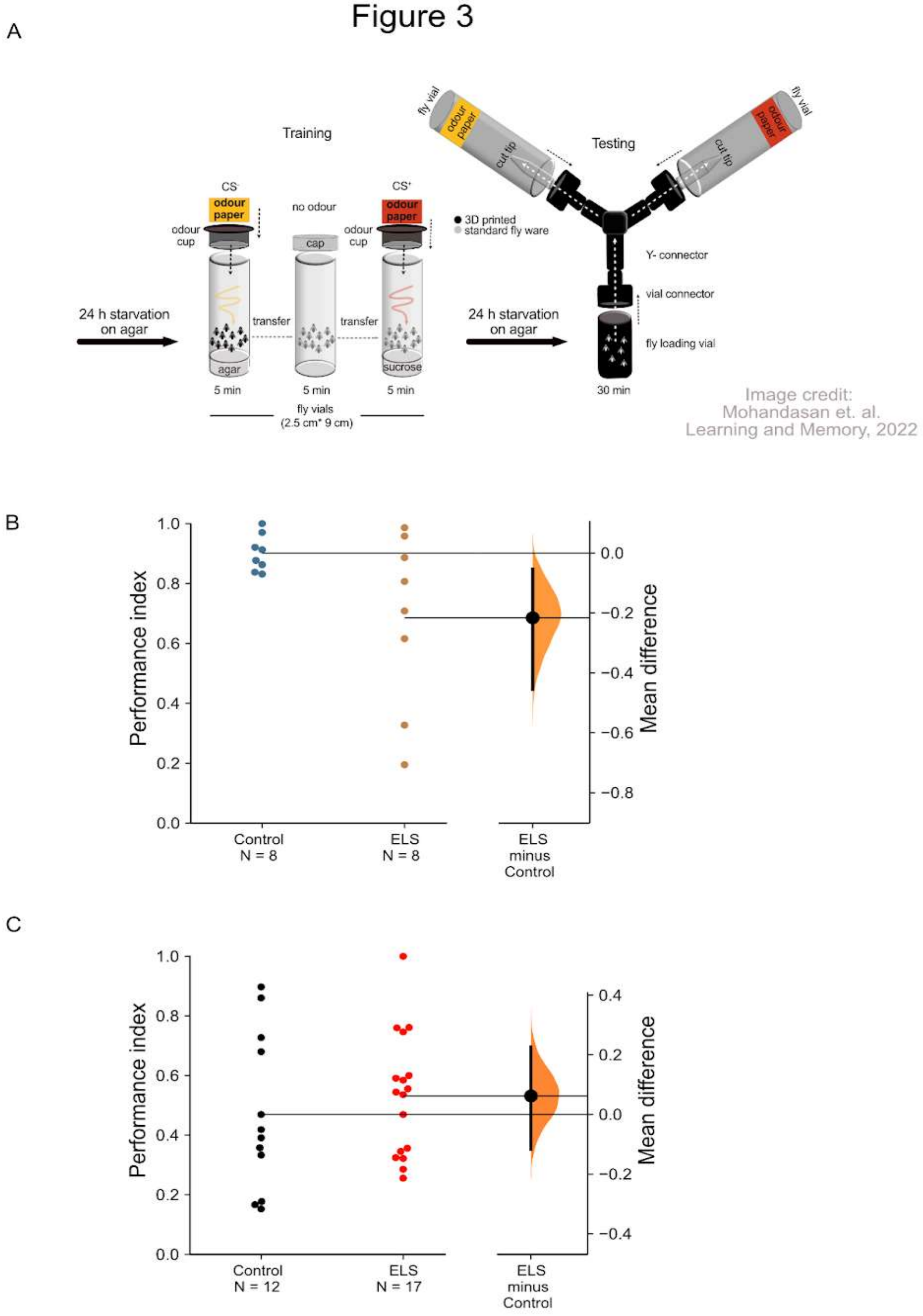


Image credit: Mohandasan et. al. Learning and Memory, 2022

Figure 3 -

A. Schematic representation for odor associated apetitive learning in flies using the Y-maze setup.

B. ELS flies showed reduced 500 mM sucrose reinforced 24 hours memory in comparison to control flies.

C. 200 mM sucrose reinforced memory was similar in ELS and control flies.

Statistics for Figure B and C were done using estimation stats. The dark dot represents the difference between the means of 2 groups. The plot shows distribution of the mean difference after performing 5000 bootstrap resampling of the data. The dark line represents 95% CI of the distribution.

## Unaltered sucrose preference in ELS flies

We investigated whether early-life starved (ELS) flies exhibit altered preference for sucrose when presented with a choice between agar-only and agar containing different concentrations of sucrose. For this we starved the flies for 24 hours and subsequently gave them 2 min choice between 0.75% agar only vs 200 mM sucrose agar/ 500mM sucrose agar.

We observed that the preference towards 500 mM sucrose was similar in both ELS and control flies (Fig 4a). We also assayed whether lower dosage of the sucrose could affect the preference of the flies. However, we observed no change in preference for 200 mM sucrose between ELS and control flies (Fig 4b)

Our results showed that ELS flies did not have any defect in assessing the reward and subsequently differentiating between different concentrations of sucrose and 0.75% agar. Hence, we suggest that the reduction in 500mM sucrose reinforced memory is not due to a defect in sensing the reward value of sucrose.

Figure 4

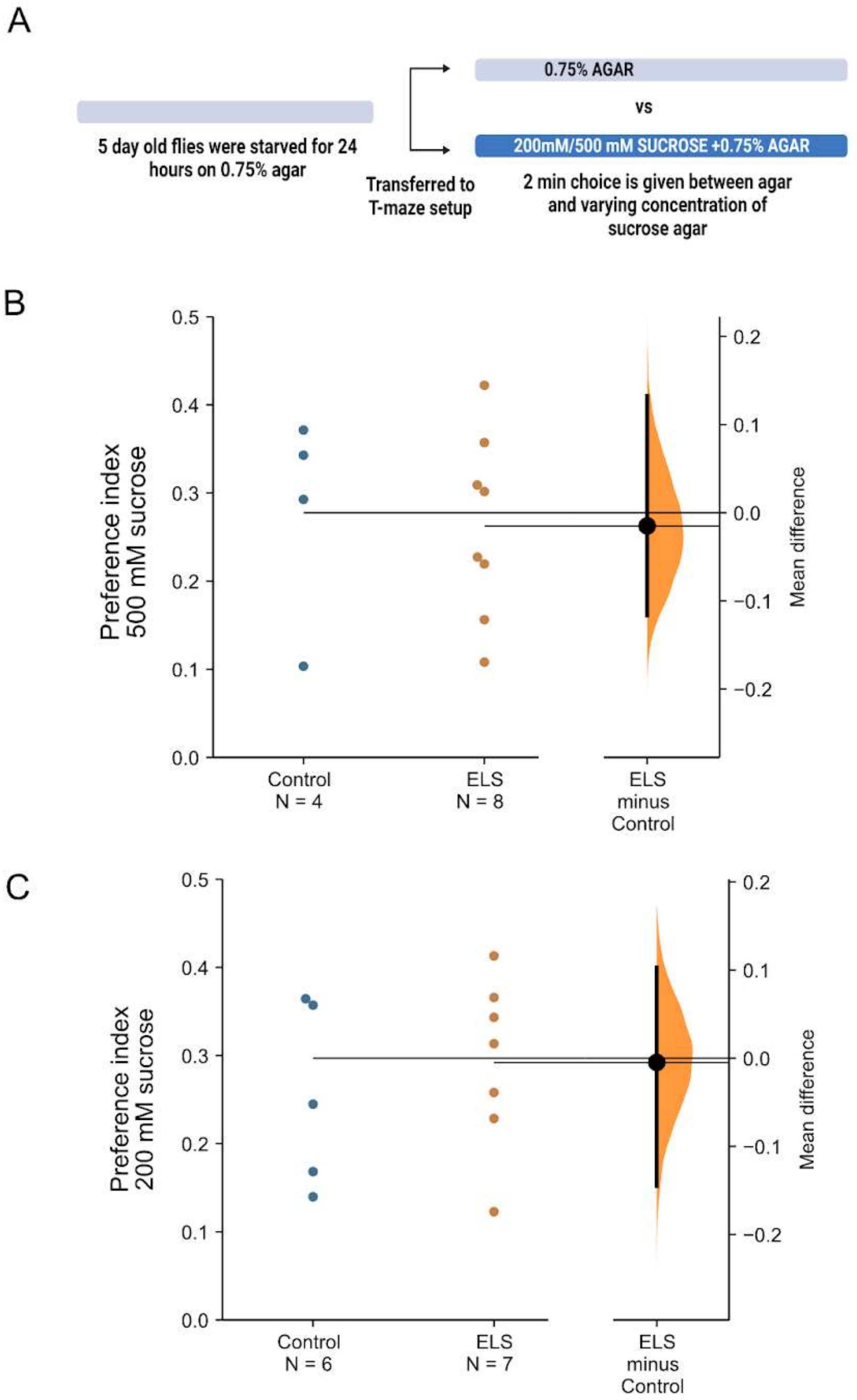

A
5 day old flies were starved for 24 hours on 0.75% agar
Transferred to T-maze setup
0.75% AGAR
vs
200mM/500 mM SUCROSE +0.75% AGAR
2 min choice is given between agar and varying concentration of sucrose agar
B
Preference index 500 mM sucrose
0.0
0.1
0.2
0.3
0.4
0.5
Mean difference
0.2
0.1
0.0
−0.1
−0.2
Control N = 4
ELS N = 8
ELS minus Control
C
Preference index 200 mM sucrose
0.0
0.1
0.2
0.3
0.4
0.5
Mean difference
0.2
0.1
0.0
−0.1
−0.2
Control N = 6
ELS N = 7
ELS minus Control

Figure 4-

A. Schematic for measuring sucrose acuity using T-mazes

B. The preference index for 500 mM sucrose were similar in both ELS and control flies.

C. The preference index for 200 mM sucrose was also similar in both ELS and control flies.

Statistics for Figure B and C were done using estimation stats. The dark dot represents the difference between the means of 2 groups. The plot shows distribution of the mean difference after performing 5000 bootstrap resampling of the data. The dark line represents 95% CI of the distribution.

## ELS flies are more susceptible to taste blunting due to exposure to high glucose diet

One of the outputs of alterations in diet is the changes that occur in taste perception of different nutrients. We measured the effect of increasing concentration of glucose in taste perception of ELS and control flies. Flies reared on 10% and 20% glucose showed reduced PER when stimulated with 5% glucose in both control and ELS (Fig 5B,E). In general both control and ELS flies reared on 10% and 20% glucose showed reduced PER upon stimulation using 5%, 10% and 20% glucose (Fig 5C,D,F and G).

ELS flies reared on 5% glucose showed enhanced PER responses when compared to control (Fig 5H). This shows that rearing flies on a high glucose diet results in a blunting effect on their sweet taste sensing.

We observed that blunting caused by 10% glucose was similar in both control and ELS flies (Fig 5I) whereas the blunting effect observed in ELS flies grown on 20% glucose showed higher blunting when compared to control flies (Fig 5J). This results is in accordance with the work of (ref) where similar taste blunting when flies are reared on high sucrose diet was seen.

Hence our results show that ELS flies are more susceptible to sensory changes occurring due to altered diet compositions.

# Figure 5

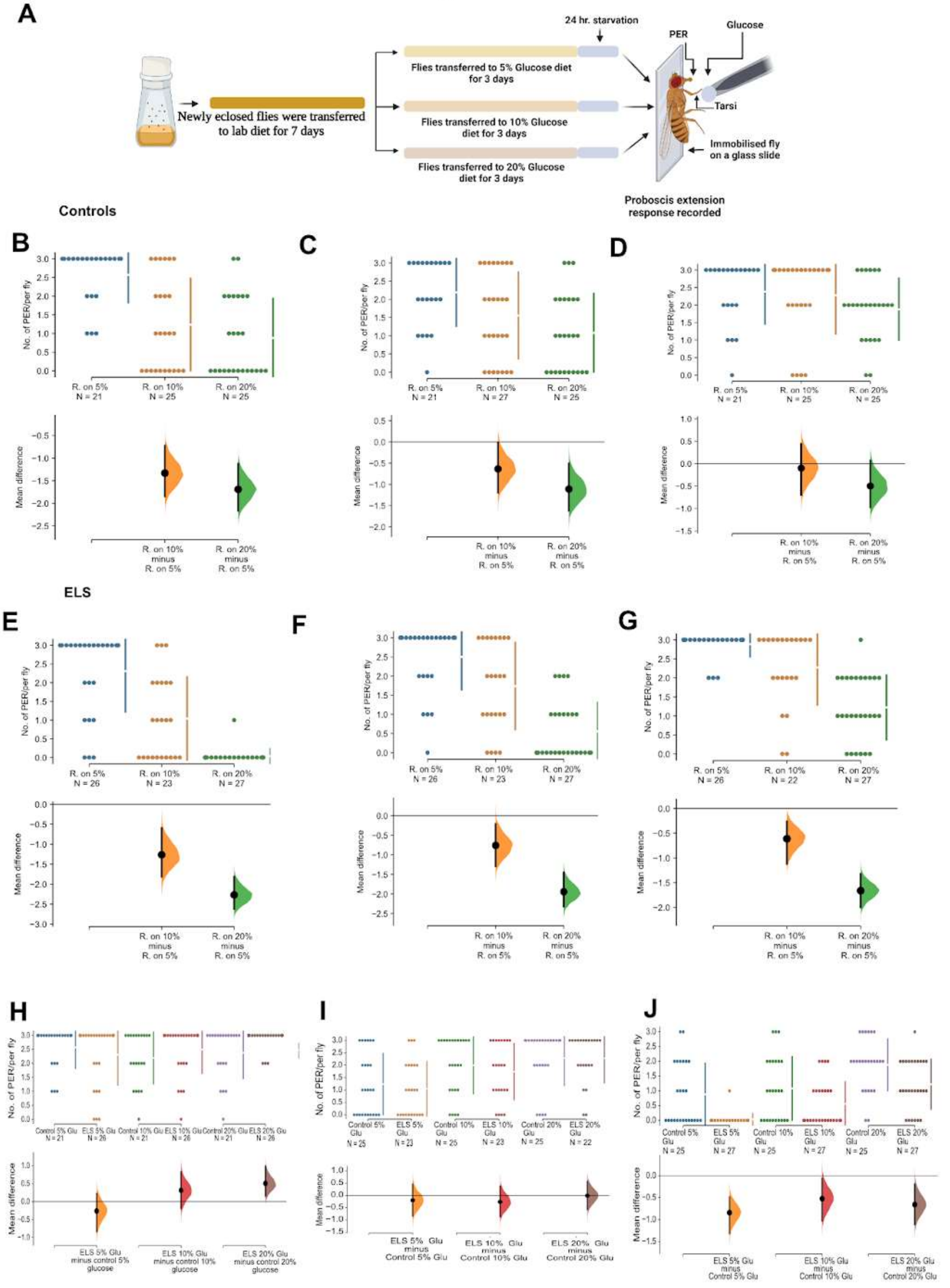

A
24 hr. starvation
PER
Glucose
Newly eclosed flies were transferred to lab diet for 7 days
Flies transferred to 5% Glucose diet for 3 days
Flies transferred to 10% Glucose diet for 3 days
Flies transferred to 20% Glucose diet for 3 days
Tarsi
Immobilised fly on a glass slide
Proboscis extension response recorded
Controls
B
C
D
No. of PER/per fly
R. on 5% N = 21
R. on 10% N = 25
R. on 20% N = 25
R. on 10% N = 27
Mean difference
R. on 10% minus R. on 5%
R. on 20% minus R. on 5%
ELS
E
F
G
R. on 5% N = 26
R. on 10% N = 23
R. on 20% N = 27
R. on 10% N = 22
H
I
J
Control 5% Glu N = 21
ELS 5% Glu N = 26
Control 10% Glu N = 21
ELS 10% Glu N = 26
Control 20% Glu N = 21
ELS 20% Glu N = 26
ELS 5% Glu minus control 5% glucose
ELS 10% Glu minus control 10% glucose
ELS 20% Glu minus control 20% glucose
Control 5% Glu N = 25
ELS 5% Glu N = 23
Control 10% Glu N = 25
ELS 10% Glu N = 23
Control 20% Glu N = 25
ELS 20% Glu N = 22
ELS 5% Glu minus Control 5% Glu
ELS 10% Glu minus Control 10% Glu
ELS 20% Glu minus Control 20% Glu
ELS 5% Glu N = 27
ELS 10% Glu N = 27
ELS 20% Glu N = 27

Figure 5-

A. Schematic for Proboscis Extension Reflex (PER) assay upon tarsal stimulation by using varying concentrations of glucose.

B. Number of PER in control flies reared on 5%, 10%, and 20% glucose when presented with 5% glucose. Flies reared on 10% and 20% glucose showed PER suppression when stimulated with 5% glucose.

C. Number of PER in control flies reared on 5%, 10%, and 20% glucose when presented with 10% glucose.Flies reared on 10% and 20% glucose showed PER suppression when stimulated with 10% glucose.

D. Number of PER in control flies reared on 5%, 10%, and 20% glucose when presented with 20% glucose. Flies reared on 5% and 10% glucose showed similar PER upon stimulation with 20% glucose. However, flies reared on 20% glucose showed PER suppression in comparison to flies reared on 5%.

E. Number of PER in ELS flies reared on 5%, 10%, and 20% glucose when presented with 5% glucose. Flies reared on 10% and 20% glucose showed PER suppression when stimulated with 5% glucose.

F. Number of PER in ELS flies reared on 5%, 10%, and 20% glucose when presented with 10% glucose. Flies reared on 10% and 20% glucose showed PER suppression when stimulated with 10% glucose.

G. Number of PER in ELS flies reared on 5%, 10%, and 20% glucose when presented with 20% glucose. Flies reared on 10% and 20% glucose showed PER suppression when stimulated with 20% glucose.

H. The PER responses for ELS flies reared on 5% glucose were higher for all three concentrations of glucose.

I. PER responses for ELS flies reared on 10% glucose showed no change for all three glucose concentrations

J. ELS flies reared on 20% glucose showed PER suppression at all three concentrations of glucose

# CONCLUSION

- We were able to successfully replicate ELS phenotype in CS-BZ flies.
- ELS flies showed reduced feeding comparative to control however when normalized by weight showed no change in feeding.
- ELS flies showed reduced 500 mM sucrose-agar reinforced memory.
- Sucrose acuity measured as a preference for 200 mM and 500 mM was similar in ELS and controls.
- Both ELS and control flies showed sweet taste blunting when reared on high concentration of glucose. However the magnitude of blunting was higher in ELS flies.

# REFERENCES

Antonow-Schlorke, I., Schwab, M., Cox, L. A., Li, C., Stuchlik, K., Witte, O. W., Nathanielsz, P. W., & McDonald, T. J. (2011). Vulnerability of the fetal primate brain to moderate reduction in maternal global nutrient availability. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(7), 3011–3016.

Aristizabal, M. J., Anreiter, I., Halldorsdottir, T., Odgers, C. L., McDade, T. W., Goldenberg, A., Mostafavi, S., Kobor, M. S., Binder, E. B., Sokolowski, M. B., & O'Donnell, K. J. (2020). Biological embedding of experience: A primer on epigenetics. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(38), 23261–23269.

Arthur, S. S., Nyide, B., Soura, A. B., Kahn, K., Weston, M., & Sankoh, O. (2015). Tackling malnutrition: a systematic review of 15-year research evidence from INDEPTH health and demographic surveillance systems. *Global Health Action*, *8*, 28298.

Astley, S., & Finglas, P. (2016). Nutrition and Health. In *Reference Module in Food Science*. unknown.

Barker, D. J., Hales, C. N., Fall, C. H., Osmond, C., Phipps, K., & Clark, P. M. (1993). Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia*, *36*(1), 62–67.

Barnes, R. H., Cunnold, S. R., Zimmermann, R. R., Simmons, H., MacLeod, R. B., & Krook, L. (1966). Influence of nutritional deprivations in early life on learning behavior of rats as measured by performance in a water maze. *The Journal of Nutrition*, *89*(4), 399–410.

Barnes, R. H., Moore, A. U., & Pond, W. G. (1970). Behavioral abnormalities in young adult pigs caused by malnutrition in early life. *The Journal of Nutrition*, *100*(2), 149–155.

Benton, D. (2010). The influence of dietary status on the cognitive performance of children. *Molecular Nutrition & Food Research*, *54*(4), 457–470.

Berkman, D. S., Lescano, A. G., Gilman, R. H., Lopez, S. L., & Black, M. M. (2002). Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. *The Lancet*, *359*(9306), 564–571.

Castro, C. A., & Rudy, J. W. (1989). Early-life malnutrition impairs the performance of both young and adult rats on visual discrimination learning tasks. *Developmental Psychobiology*, *22*(1), 15–28.

Champakam, S., Srikantia, S. G., & Gopalan, C. (1968). Kwashiorkor and mental development. *The American Journal of Clinical Nutrition*, *21*(8), 844–852.

Cravioto, J., & Robles, B. (1965). EVOLUTION OF ADAPTIVE AND MOTOR BEHAVIOR DURING REHABILITATION FROM KWASHIORKOR. *The American Journal of Orthopsychiatry*, *35*, 449–464.

Dauncey, M. J., & Bicknell, R. J. (1999). Nutrition and neurodevelopment: mechanisms of developmental dysfunction and disease in later life. *Nutrition Research Reviews*, *12*(2), 231–253.

Dobbing, J. (1970). Undernutrition and the developing brain. The relevance of animal models to the human problem. *American Journal of Diseases of Children* , *120*(5), 411–415.

Dobbing, J., & Sands, J. (1973). Quantitative growth and development of human brain. *Archives of Disease in Childhood*, *48*(10), 757–767.

Fan, X., Wang, L., Wei, X., Zhang, J., Su, X., Cui, L., & Wang, Z. (2021). The impairment of continuous malnutrition on larval fish swimming performance at the mouth-opening stage. *Aquaculture* , *544*, 737053.

FrankovÁ, S., & Barnes, R. H. (1968). Effect of Malnutrition in Early Life on Avoidance Conditioning and Behavior of Adult Rats1. *The Journal of Nutrition*, *96*(4), 485–493.

Friedrich, M. J. (2015). Gut Bacteria Combined With Poor Diet May Promote Childhood Malnutrition. *JAMA: The Journal of the American Medical Association*, *313*(15), 1506–1506.

Galler, J. R. (1984). Behavioral Consequences of Malnutrition in Early Life. In J. R. Galler (Ed.), *Nutrition and Behavior* (pp. 63–117). Springer US.

Galler, J. R., Bringas-Vega, M. L., Tang, Q., Rabinowitz, A. G., Musa, K. I., Chai, W. J., Omar, H., Abdul Rahman, M. R., Abd Hamid, A. I., Abdullah, J. M., & Valdés-Sosa, P. A. (2021). Neurodevelopmental effects of childhood malnutrition: A neuroimaging perspective. *NeuroImage*, *231*, 117828.

Galler, J. R., Ramsey, F., Solimano, G., Lowell, W. E., & Mason, E. (1983). The influence of early malnutrition on subsequent behavioral development. I. Degree of impairment in intellectual performance. *Journal of the American Academy of Child Psychiatry*, *22*(1), 8–15.

Grantham-McGregor, S. (1995). A review of studies of the effect of severe malnutrition on mental development. *The Journal of Nutrition*, *125*(8 Suppl), 2233S – 2238S.

Green, C. J. (1999). Existence, causes and consequences of disease-related malnutrition in the hospital and the community, and clinical and financial benefits of nutritional intervention. *Benefits of Nutritional Support in Hospitals and the Community*, *18*, 3–28.

Griffiths, W. J., Jr, & Senter, R. J. (1954). The effect of protein deficiency on maze performance of domestic Norway rats. *Journal of Comparative and Physiological Psychology*, *47*(1), 41–43.

Hales, C. N., & Barker, D. J. P. (2001). The thrifty phenotype hypothesis: Type 2 diabetes. *British Medical Bulletin*, *60*(1), 5–20.

Hannah Simmons, M. S., & Deepthi Sathyajith, M. P. (2018, May 22). *Drosophila as a Model Organism*. News-Medical.net. https://www.news-medical.net/life-sciences/Drosophila-as-a-Model-Organism.aspx

Hillman, D. E., & Chen, S. (1981). Vulnerability of cerebellar development in malnutrition-I. Quantitation of layer volume and neuron numbers. *Neuroscience*, *6*(7), 1249–1262.

Kahsai, L., & Zars, T. (2011). Learning and memory in Drosophila: behavior, genetics, and neural systems. *International Review of Neurobiology*, *99*, 139–167.

Kallen, D. J. (1973). *Nutrition, Development, and Social Behavior: Proceedings*. U.S. National Institutes of Health.

Levitsky, D. A., & Barnes, R. H. (1973). Malnutrition and animal behavior. *Nutrition, Development and Social Behaviour*, 3–16.

*Malnutrition*. (n.d.). Retrieved June 15, 2023, from https://www.who.int/health-topics/malnutrition#tab=tab_3

Manteca, X. (2011). Nutrition and behavior in senior dogs. *Topics in Companion Animal Medicine*, *26*(1), 33–36.

Marcos, A. (2000). Eating disorders: a situation of malnutrition with peculiar changes in the immune system. *European Journal of Clinical Nutrition*, *54 Suppl 1*, S61–S64.

Massaro, T. F., Levitsky, D. A., & Barnes, R. H. (1974). Protein malnutrition in the rat: its effects on maternal behavior and pup development. *Developmental Psychobiology*, *7*(6), 551–561.

Ngo, J., Ortiz-Andrellucchi, A., & Serra-Majem, L. (2016). *Malnutrition: Concept, Classification and Magnitude*. https://doi.org/10.1016/B978-0-12-384947-2.00439-6

Olivares, G. H., Núñez-Villegas, F., Candia, N., Oróstica, K., González-Ramírez, M. C., Vega-Macaya, F., Zúñiga, N., Molina, C., Oliva, C., Mackay, T. F. C., Verdugo, R. A., & Olguín, P. (2023). Early-life nutrition interacts with developmental genes to shape the brain and sleep behavior in Drosophila melanogaster. *Sleep*, *46*(4). https://doi.org/10.1093/sleep/zsad016

Papathakis, P. C., Singh, L. N., & Manary, M. J. (2016). How maternal malnutrition affects linear growth and development in the offspring. *Molecular and Cellular Endocrinology*, *435*, 40–47.

Patil, S. G., Sekhar, S., Agarwal, A., Oviya, T. S., Rout, D., & Megha. (2022). Impact of late larval nutritional stress on adult metabolic, gut and locomotor phenotypes in Drosophila melanogaster. In *bioRxiv* (p. 2022.06.30.498321). https://doi.org/10.1101/2022.06.30.498321

Prado, E. L., & Dewey, K. G. (2014). Nutrition and brain development in early life. *Nutrition Reviews*, *72*(4), 267–284.

Roger, K., Vannasing, P., Tremblay, J., Bringas Vega, M. L., Bryce, C. P., Rabinowitz, A. G., Valdés-Sosa, P. A., Galler, J. R., & Gallagher, A. (2022). Impact of Early Childhood Malnutrition on Adult Brain Function: An Evoked-Related Potentials Study. *Frontiers in Human Neuroscience*, *16*, 884251.

Saunders, J., & Smith, T. (2010). Malnutrition: causes and consequences. *Clinical Medicine*, *10*(6), 624–627.

Shetty, P. (2006). Malnutrition and undernutrition. *Medicine*, *34*(12), 524–529.

Shih, M.-F. M., Davis, F. P., Henry, G. L., & Dubnau, J. (2019). Nuclear Transcriptomes of the Seven Neuronal Cell Types That Constitute the Drosophila Mushroom Bodies. *G3* , *9*(1), 81–94.

Spindler, S. R., & Hartenstein, V. (2010). The Drosophila neural lineages: a model system to study brain development and circuitry. *Development Genes and Evolution*, *220*(1-2), 1–10.

Stratton, R. J., Green, C. J., & Elia, M. (2003). *Disease-related Malnutrition: An Evidence-based Approach to Treatment*. CABI.

*The UNICEF/WHO/WB Joint Child Malnutrition Estimates (JME) group released new data*. (n.d.). Retrieved June 15, 2023, from https://www.who.int/news/item/31-03-2020-unicef-who-wb-jme-group-new-data

Tu, M.-P., & Tatar, M. (2003). Juvenile diet restriction and the aging and reproduction of adult Drosophila melanogaster. *Aging Cell*, *2*(6), 327–333.

Upadhyay, S. K., Agarwal, K. N., & Agarwal, D. K. (1989). Influence of malnutrition on social maturity, visual motor coordination & memory in rural school children. *The Indian Journal of Medical Research*, *90*, 320–327.

Winick, M. (1975). Nutrition and Brain Development. In G. Serban (Ed.), *Nutrition and Mental Functions* (pp. 65–73). Springer US.

Xu, J., He, G., Zhu, J., Zhou, X., St Clair, D., Wang, T., Xiang, Y., Zhao, Q., Xing, Q., Liu, Y., Wang, L., Li, Q., He, L., & Zhao, X. (2014). Prenatal nutritional deficiency reprogrammed postnatal gene expression in mammal brains: implications for schizophrenia. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum* , *18*(4). https://doi.org/10.1093/ijnp/pyu054

Zúñiga-Hernández, J. M., Olivares, G. H., Olguín, P., & Glavic, A. (2023). Low-nutrient diet in Drosophila larvae stage causes enhancement in dopamine modulation in adult brain due epigenetic imprinting. *Open Biology*, *13*(5), 230049.