# Maternal Nutritional Influence on Progeny Physiology, Longevity, and Immune System in *Drosophila melanogaster*

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

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Bonn 2024

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät

der Rheinischen Friedrich-Wilhelms-Universität Bonn

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Tag der Promotion: 17.02.2025

Erscheinungsjahr: 2025

## Declaration

The present thesis has not been proposed for any other degree or proficient qualification and is the yield of my independent investigations.

Candidate: Seyhmus Bayar

## Abstract

Maternal obesity can influence the health of offspring through immunity, epigenetic regulations, telomeres length and development of gut dysbiosis, cancer, diabetes and brain disorders, which may also affect the long term and result in diminished life expectancy. *Drosophila melanogaster* is a practical model for investigating the role of maternal diet in descendants. The primary immune cells of invertebrates are named hemocytes, and the majority of hemocytes consist of plasmatocytes, which are macrophage-like cells. Genetic, epigenetic, or non-optimal maternal circumstances can cause the hemocytes of descendants to adapt and reprogram their development *in Drosophila*, resulting in long-term influences.

It is unknown how the dietary source of maternal obesity longitudinally influences progeny health and the innate immune system. To this end, a maternal obesity model was generated using *Drosophila*. Briefly, virgin female flies were fed with a control diet (CD), a high-sugar diet (HSD), and a high-fat diet (HFD) for 7 days, and all groups mated on CD with CD-fed males. The progeny's body weight, lipidomics, genetic regulations, longevity, and locomotor outcomes were longitudinally investigated on days 0, 10, and 50. Moreover, light-induced axonal degeneration was investigated in progeny to understand whether maternal diet can impact neurodegeneration.

Both HSD and HFD mothers reduced the body weight and caused the upregulation of various lipid species. While the body weight of maternal high-fat diet (HFDm) and high-sugar diet (HSDm) females stayed steady on days 0 and 10 and declined on days 50 compared to CDm, the body weight of males fluctuated between time points. The axonal degeneration was raised solely in the male progeny in the HFDm, and the bulk RNA-seq analysis revealed uniquely downregulated genes related to axonogenesis and eye development terms. Only males in the HFDm group had diminished longevity, and hemocyte-specific NF-kB knockout worsened longevity. However, challenges with bacteria did not reveal any disparity.

In summary, maternal HFD and HSD result in shared and unique phenotypes in the progeny in a sex-specific manner. Further investigations should target hemocyte-specific knock-in lines, and challenge flies with different types of pathogens to reveal the exact mechanism.

Keywords: Maternal diet, metabolism, immune system, plasmatocytes, longevity.

## Acknowledgements

First of all, I would like to thank my advisor, Prof. Elvira Mass, for her excellent guidance, continuous support, and advice for my academic development throughout my PhD.

I want to thank The Ministry of National Education of the Republic of Turkey, which honoured me with the doctoral scholarship given to excellent students in their field following a diligent examination procedure.

I am grateful for to all AG Mass members, especially Dr. Nelli Blank, Dr. Hao Huang, Kerim Acil and Cornelia Cygon, for their extraordinary academic sharing and cooperation.

I am thankful to Karolina Doubkova, Lea Seep, Dr. Jelena Zurkovic, Lennart Gollenbeck, Dr. Aurelie Muria and Dr. Anshupa Sahu for their helps.

I am thankful to Prof. Gaia Tavosanis and Prof. Michael Heneka for participating in my thesis advisory committee and guiding my academic development.

I thank all LIMES Institute professors, especially Drosophilists, and technical employees for their continuous supports.

I am thankful to my previous advisor Prof. Gülgün Sengül for her contributions to my academic development.

Finally, I want to thank my wife, Zeynep Ceylan-Bayar, mother, Sara Bayar, father, Mehmet Bayar, and siblings for always encouraging and supporting me.

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## Abbreviations

CD: Control diet

HFD: High-fat diet

HSD: High-sugar diet

CDm: Maternal control diet

HFDm: Maternal high-fat diet

HSDm: Maternal high-sugar diet

MyD88: Myeloid differentiation primary response 88

JAK/STAT: The Janus kinase signal transducer and activator of STAT

Relish: Drosophila NF-kB transcription factor Drosophila harnesses an intricate array of PRR: Pattern Recognition Receptor

TLR Toll-like receptor

PGRP: Peptidoglycan recognition protein

GNBP: Gram-negative binding protein

IMD: Immune Deficiency

*E. coli*: Escherichia coli

AMP Antimicrobial Peptide

IL-6: Interleukin-6

TNF-α: Tumour necrosis factor-alpha

DAG: Diacylglycerol

TAG: Triacylglycerol

CE: Cholesterol ester

ST: Cholesterol

PS: Phosphatidylserine

PE-O: Phosphatidylethanolamine ether

HexCer: Hexosyl ceramide,

PC-O: Phosphatidylcholine ether

LPC: Lysophosphatidylcholine

Cer: ceramides

LPE: lysophosphatidylethanolamine

SM: sphingomyelin

PG: Phosphatidylglycerol

PE. Phosphatidylethanolamine

PUFA: Poly-unsaturated fatty acid

PA: Phosphatidic acid

PI: Phosphatidylinositol,

PIPOS: Phosphatidylinositol phosphates

LPCH: Lysophosphatidylcholines

MAG Monoacylglycerol

HSC: Hematopoietic stem cells

KEGG: Kyoto Encyclopedia of Genes and Genomes

GO: Gene ontology

# Chapter 1: Introduction

## 1.1 Epidemiology of obesity

Obesity is one of the primary pandemic epidemics endangering individuals and their well-being[1]. An enormous formation of fat that typically results in being overweight and an elevated body mass index is referred to as obesity[2]. Based on the existing research, obesity and its associated problems impact not only the individual but also their offspring[3], which raises death and morbidity rates, as well as societal expenses for healthcare associated morbidity[1]. Hence, obesity is a phenomenon that causes significant consequences on the socioeconomic scale beyond being a matter of health. Multiple genetic[4], behavioral[5] and environmental[6] factors promote the epidemiology of obesity. To combat such a worldwide health burden, multidisciplinary and efficient actions at multiple tiers of governments, medical professionals, communities, and professionals are required. This could be accomplished by addressing the underlying causes and carrying out evidence-based strategies that lower the burden of obesity while improving public health.

Since 1980, the prevalence of overweight people has duplicated worldwide, and almost a third of the global case has been characteristics of obesity[7]. The World Health Organization (WHO) estimates that in 2016, there were over 600 million obese individuals and almost 2 billion overweight adults worldwide[8]. In addition to the substantial proportion of overweight in the population in general, there has been a substantial rise in obesity, especially in females. The density of age-matched obese adults was 42.4%, and there was no statistical distinction between females and males in 2017–2018, however, the severity of obesity was 9.2% higher in women in the United States[9].

Germany is one of the most severely influenced land by obesity[10]. The Gutenberg Health Study (GHS) demonstrated a link between body mass index (BMI), type 2 diabetes, insulin resistance, elevated triglycerides, low HDL cholesterol, arterial hypertension, and cardiovascular disease. The study also revealed that 48.1% of males and 32.1% of females are overweight, and 26.3% of males and 24.1% of females are obese in the Mainz-Bingen locations[11].

In summary, the incidence and prevalence of obesity rising in the world due to a complicated interplay between changes in the dietary environment and genetic, epigenetic, physical, social and environmental factors[12]. To this end, obesity is one of the most crucial phenomena that must be overcome for the sustainability of public health and economic development in both developed and developing countries.

### 1.2 Molecular characteristics of obesity

Obesity results in expanded body, organ and cell size and consequently the body size-related parameters such as BMI, lipid accumulation index, body roundness index, waist-to-height ratio, and visceral adiposity index are linked to the development of obesity, which can also boost the risk of type 2 diabetes[13]. Obesity has a remarkable effect on the liver epididymal and fat tissues, raising rubidium, iron, and selenium levels compared to the control group[14]. Several novel proteins and metabolites, including the urokinase plasminogen activator receptor, fibroblast growth factor 23, thrombospondin-2, proteinase-3, or interleukin-18, carnitines, cinnamoyl glycine, indole 3-propionate, eicosapentaenoic acid, and threonine, have been identified as potential molecular fingerprints of susceptibility to bodyweight gain in young adults with over two decades of follow-up into mid-life[15]. Some proteomic biomarkers of obesity are involved in metabolic pathways, oxidative stress, chaperone family and inflammation proteins, which also may be dysregulated in obesity-related disorders and serve as biomarkers for the development of comorbidities and the progression of obesity[16].

Moreover, obesity consequences from raised adiposity include growing fat cells and worsening insulin and leptin metabolism, which is also associated with higher inflammation and some metabolic disorders, including type 2 diabetes, non-alcoholic fatty liver disease, cardiovascular disease, and some cancers[17]. Bioinformatics analysis revealed that serpin peptidase inhibitor clade H member 1 (SERPINH1), the signal transducer and activator of transcription 3 (STAT3), coronin 1C (CORO1C), major vault protein (MVP), eukaryotic translation elongation factor 1 gamma (EEF1G), integrin subunit beta 5 (ITGB5), pericentriolar material 1 (PCM1), sirtuin 1 (SIRT1), ribosomal protein S2 (RPS2), and phosphatase and ptensin homolog (PTEN) genes can include in the pathology and prognosis of obesity[18].

TrkB–BDNF signaling is essential for neuronal morphology, plasticity, and survival, and the leptin-melanocortin pathway, which functions in mammalian nutrition intake, plays a role in the development of obesity[19]. Hyperinsulinism, lesions in the ventromedial side of the hypothalamus, perceived levels by hypothalamic neuron groups, hereditary factors, and dysregulation of the serotoninergic and histaminergic systems are among the primary factors attributing to the etiopathology of obesity[20,21].

Pursuant to its description, obesity is a condition of systemic inflammation[22] that promotes the risk of high blood pressure[23] and elevated glucose levels. It also boosts the potential for inflammatory immune factors[24] since obesity gradually activates innate immune cells or pathways.

Moreover, obesity correlated with a raised macrophage infiltration[2]. Multiple investigations discovered an alteration in the immune cells functions from an anti-inflammatory state to a pro-inflammatory state in obesity[1]. Apart from contributing to immune system dysregulation[25], obesity can lead to metabolic syndromes[26], hormonal obstacles[2], respiratory[27], digestive, and cardiac conditions[28], besides hypertension and anomalies in the reproductive system. Further diseases like diabetes[29], cancer[30], disorders of the nervous system[20] and the liver[31] may ensue from this.

In summary, although the exact causes of co-morbidities of obesity are still unknown, dysregulations of genetic factors, glucose metabolism, inflammatory pathways, and fat turnover may be essential factors [32].

### 1.3 Maternal obesity

Besides the individual detrimental impacts, obesity-related changes in metabolism might also have a bearing on epigenetic processes through DNA methylation, building an individualized epigenetic program vital to healthy embryonic development and potentially influencing the health of the progeny by way of early or later valuable effects[6,33].

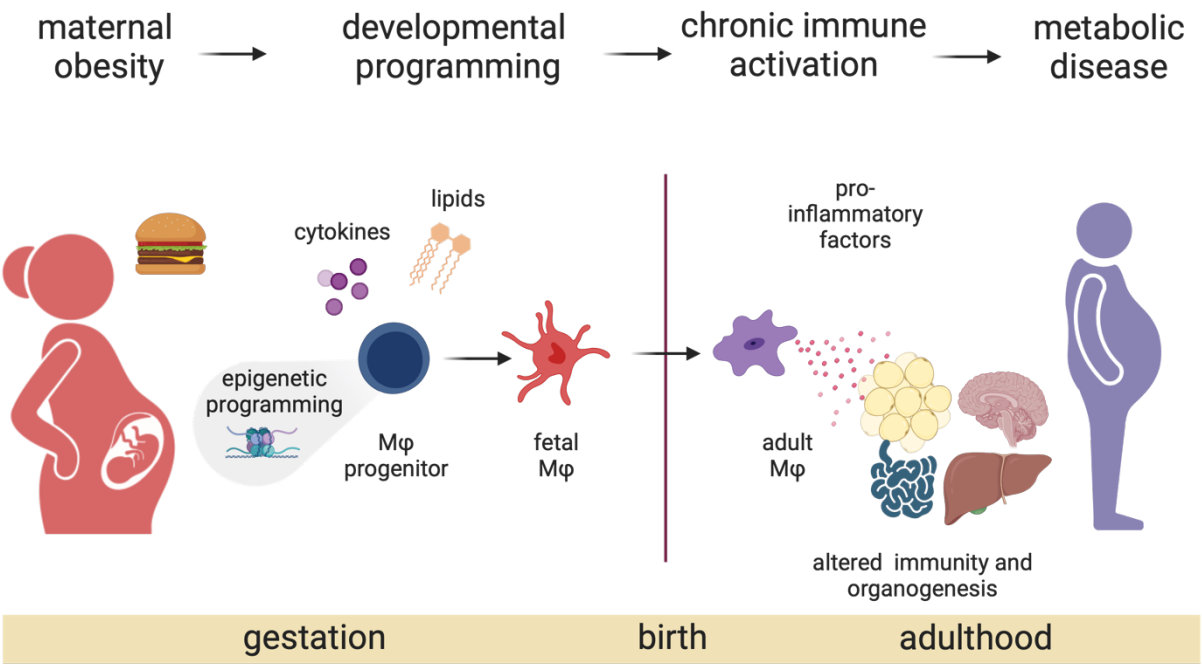



**Figure 1: Maternal obesity and reprograming of immune system.** Metabolic and epigenetic modifications can reprogram fetal macrophages, influence development of the immune system and organogenesis and having long-term impacts on offspring. (figure created using the biorender.com).

Evidence so far demonstrates the long-term consequences of maternal diet on the offspring's health and development[3]. Epigenetic[34] and immune modifications due to obesity[35,36] may lead to the reprogramming of fetal macrophages[36], which may affect organogenesis and the immune system during adulthood[37–39]. Consequently, reprogramming of fetal macrophages may have long-lasting effects on the health of the progeny. HFD is one of the considerably used food sources in the induction of maternal obesity. Another essential food source used in experimental obesity models, HSD, is a high-calorie diet source used to induce obesity and metabolic syndromes[40–44].

Calorie-rich diets can trigger obesity and chronic inflammation, which might then have a consequence on the progeny's health during gestation and development by boosting lipotoxicity[45], inflammatory cytokines[46], or influencing epigenetic modifications[47]. Experimental investigations have shown evidence for the causative consequences of

maternal obesity on descendant outcomes, which are thought to be mediated, at least in part, by modifications in epigenetic processes such as DNA methylation and possibly by changes in the gut microbiome[3].

Based on the hypothesis of prenatal programming, the fetus' adaptations to suboptimal intrauterine environmental conditions essentially turn into reprogramming this environment. It has become widely accepted that the fetus' cell counts and metabolic processes are impacted by unsuitable intrauterine environments[22,36,39,48,49]. Moreover, obesity has been proven to cause predisposition to prenatal and postnatal developmental anomalies in the progeny, including preterm birth, early death, antenatal macrosomia, birth anomalies[26,50,51]. It is conceivable to inherit these traits from one or both of your parents. Maternal obesity is likely to impact progeny drastically since the major part of development takes place within the mother's environment. Similarly, embryo influenced by its parents' genetic traits through prenatal development, which unfolds in the womb[4,52]. Consequently, environmental changes in this environment might directly or indirectly affect fetal growth and development through epigenetics from a metabolic perspective.

While there are vast health issues that result from maternal obesity in progeny, studies have reported that the findings obtained might result in the central nervous system malfunctioning in progeny at both an early and late period[3,33,53–55].

Progeny health might have been impacted by elevated lipotoxicity, inflammatory cytokines, or epigenetic modifications if obesity and chronic inflammation remain persistent during pregnancy and infant feeding[39,56–58]. Maternal obesity might impact the characteristics and activity of immune cells, increasing their activation mediated the expression of inflammatory mediators and the presence of raised lipid accumulation[48,58].

Several types of central nervous system anomalies[27,55,59–62] can be attributed to maternal weak glucose intolerance[21], which can boost the probability of neural tube defects[54,62] in offspring. Furthermore, it is possible that maternal obesity impacts neuroinflammation[59] processes through the activation of microglial[63] and perivascular macrophages[64], which are caused by the production of reactive oxygen species[65] and pro-inflammatory cytokine[22]. Further investigation has discovered that the downregulation of BDNF in the hippocampus promotes changes in serotonin and dopaminergic dissemination leading to nervous system deterioration and this function[49,63,66]. Moreover, the cerebrovasculature diminishes as the number of Tau-

positive neurons and Aβ plaque load rise, compromising cognitive function and long-term memory[53,56,67]. These factors may affect the neuronal and axonal fitness of the progeny and increasing their susceptibility to various brain diseases.

Additionally, a strong positive link was observed between placental macrophage populations and foetal microglia, which led to a reduced resting phenotype and impaired phagocytosis[27,60,61]. Pre-pregnancy obesity blunts the NF-κB signaling, alters the capability of the blood monocytes in the umbilical cord, and worsens the clearing of viral and bacterial pathogens[36]. Furthermore, maternal obesity-related dysfunctional vasculature activates CD68 and CD3-positive immune cells, simultaneously diminishing placental endothelial cells[61,68].

Previous investigations have also indicated links between maternal obesity and parameters that can affecting life expectancy, such as telomere length, which are key components of longevity. For example, a study revealed an association between the length of telomeres in newborns and mothers' BMI[69]. Another study demonstrated that embryos of obese mice can have dysfunction in their telomers[70].

Sexual dimorphism emphasizes the significance of using both females and males in diet-related investigations. For example, a study revealed that the maternal high-fat diet could have a broader effect on gene expression in the male progeny than the females, driving impaired insulin and glucose levels and raising the weights of the kidneys and livers[71].

To conclude, maternal obesity can cause dysbiosis, genetic, epigenetic, and metabolic and immune regulations that can impact the development and overall health of the progeny and also influence lifespan.

### 1.4 The influence of maternal obesity on the innate immune system

Given the level of complexity, obesity is a metabolic illness characterized by several underlying causes and contributes to a significant global epidemic. Related recent studies, however, have demonstrated the link between obesity and maternal health, where obesity represents a significant risk in and of itself. The complications of maternal obesity stretch beyond the regulation of body weight, impacting both the mother and her progeny[3,72,73]. A better population health profile is thereby rendered possible by the intricate interactions between obesity, maternal health, and the innate

immune system, which may uncover underlying processes alongside potential therapeutic paths.

The immune system provides a vital role in regulating metabolic homeostasis[74], and the development of adiposity[32] processes is highly associated with immune system instability. Obesity is defined by prolonged low-grade inflammation and elevated levels of pro-inflammatory substances like interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α)[48]. Systemic insulin resistance and metabolic illnesses are induced by these variables disrupting the insulin signaling pathway[45,50,57]. Adipose tissue has numerous purposes, including that of a storage organ[2,75]. It also functions as an immune-mediated centre, containing various immune cell types that can cause inflammation and dysregulation of the metabolism, comprising macrophages, T cells, and ILCs permanently resident in adipose tissue[46].

Given its substantial impact on the intrauterine environment, maternal adipose tissue is the cause of variability in perinatal growth and the predisposition to metabolic diseases in the progeny in later life[76]. Recent evidence indicates that maternal obesity increases the probability of prolonged placental inflammation, which leads to prenatal overgrowth as well as irregular food transport and inadequate metabolic programming[22]. Likewise, it maintains inflammation, modifies the environment of adipokines and cytokines interacting with the fetus, and raises the progeny's risks of obesity, insulin resistance, and cardiovascular diseases[39,61,77].

As the initial line of protection against pathogens, the immune system's natural response reacts by detecting metabolic stress and initiating inflammation responses[78,79]. Maternal obesity-related dysregulation of the innate immune system increases placental inflammation[22], compromising prenatal metabolic programming and nutrition exchang[49,80]. Amongst such is metabolic stress, which induces an intense and quick inflammatory response. Insulin resistance and local inflammation are made severe by the pro-inflammatory phenotype of macrophages that infiltrate adipose tissue and the placenta as the consequence of adiposity-induced chemokine signaling[46,75,81]. Adipose tissue-resident macrophages play a highly significant role in coordinating the inflammation and metabolic dysfunction that are specific to this tissue; as adiposity rises, they reflect a phenotypic shift from an anti-inflammatory to a pro-inflammatory state[75,82]. Interplay of adiposity with maternal health and the innate

immune system have revealed novel therapeutic interventions and preventive strategies[2].

The novel possibility of targeting these critical mediators of innate immune activation holds some promise in alleviating adiposity-related inflammation and heralding new therapeutic interventions for the adverse impacts on progeny‘s health state[45]. In addition, lifestyle intervention, including diet modification and appropriate guidance on exercise[68], is considerable not just during pregnancy but also prior to conception as a means of reducing the incidence of heritage on the descendants’ metabolic health[4,30]. This means that maternal adiposity represents one of the significant public health challenges in medicine today. The innate immune system is not only a significant driver in the pathogenesis of these conditions but also in the propagation of such conditions across generations [83]. This, therefore, emphasizes the need to decipher the mechanisms for identifying pathways through which innate immune activation influences maternal adiposity and can fulfil the rationale for developing targeted manipulations in breaking the cycle of adiposity to boost the health of both the mother and the progeny.

### 1.5 *Drosophila melanogaster* as a maternal obesity model

*Drosophila* is considered a worthy and convenient model organism for contemporary biology research in metabolism[41,42,47,84,85], development[42], and immunity[86–88]. More recently, its value has also been appreciated in studies on maternal obesity and correlates of this condition in the progeny[30,47]. Despite significant evolutionary distances between flies and mammals, many key fundamental biological processes, such as lipid metabolism, insulin signaling, and innate immunity, have been highly conserved in the two species[89]. [34–36]

To attempt to investigate the molecular mechanisms underpinning obesity and its adverse impact on offspring *Drosophila melanogaster* has evolved into a highly effective and helpful model organism to revealed its implications for progeny needs and the subsequent metabolic and epigenetic influence to combat metaflammation[47,52,85].

### 1.5.1 Innate immune system in *Drosophila melanogaster*

Drosophila is an efficient and useful model organism for immunological research due to its short life cycle and the structure and functions of the innate immune system it preserves.[87,90,91]. Drosophila has similarities to mammalian in immune ontogenies, dynamics of proteins, receptor functionality of receptors, and signaling pathway that regulate the innate immune response in *Drosophila*.[92,93]. Moreover, *Drosophila*, has an extensive immune system and similar analogies to vertebrates, is suited to serve as a model organism to comprehend the structure and function of host defence mechanisms[92].

Hemocytes and fat body play the key role in immunologic functions of *Drosophila*. Hemocytes play a crucial innate function for arranging an immune response. The fat body, which in the fly is an analogous organ to the mammalian liver and adipose tissue, is central for immune functions[86,94]. Current investigations demonstrate different hemocyte subpopulations and their multiple functions in host defence[94]. Essentially, the key immune-related effector cells in *Drosophila* are termed plasmatocytes, which are analogous to mammalian macrophages. Similar to mammalian macrophages, plasmatocytes are responsible for phagocytosis of pathogens and dead cells, and restoring processes connected to wound resolution[90,95,96]. The main objective of the specialized hemocytes is melanization, regarded as a crucial safeguard against infections from parasites and wound containment[97]. In response to immunological challenges, lamellocytes help to produce and shed the contents of the extracellular matrix, allowing them to repair scars and confine foreign aggressors[98]. The crystal cells in plays role in melanotic encapsulation of pathogens through initiating the enzymatic and secretory actions of melanin precursors[99].

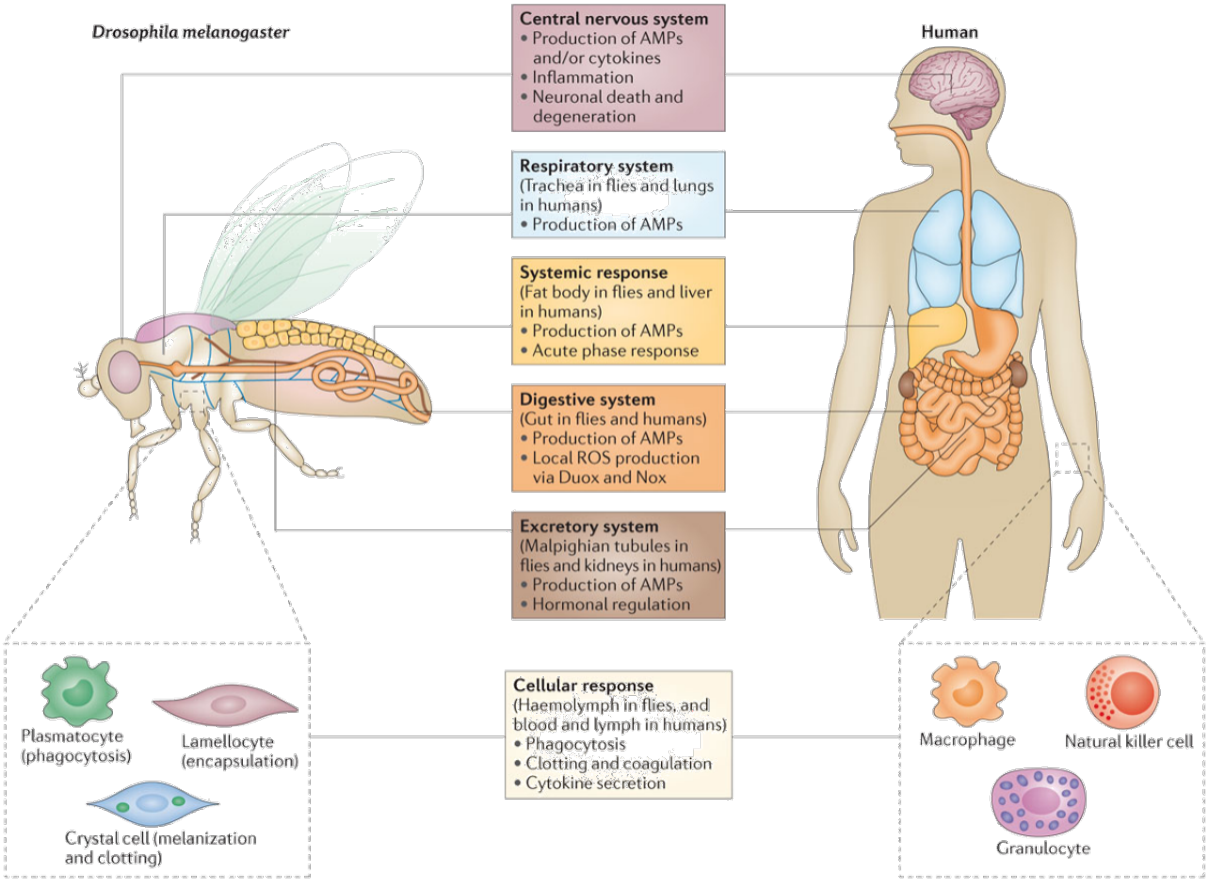



**Figure 2: Organs and immune cells of *Drosophila*.** Despite being tiny, *Drosophila* have organs and systems and cells that has similarpities to humans such as central nervous system, respiratory system, fat body, that functions as a liver and adipose tissue, digestive system, and excretory system. *Drosophila* also have immune cells similar to those of humans. The primary immune cells are the *Drosophila melanogaster* plasmatocytes, crystal cells, and lamellocytes. The figure was taken from Buchon N., Silverman N and Cherry S., 2014[89].

The Pattern Recognition Receptors (PRRs) of *Drosophila* are various and intricate, sensing PAMPs from pathogens, including Toll-like, Peptidoglycan recognition proteins, and Gram-negative binding proteins[100]. Antimicrobial Peptides (AMPs) are an extensive class of molecules wholly synthesized and secreted once bioactive immune signaling pathways. Especially, Toll and Immune Deficiency (IMD), including Defensin, Diptericin, and Drosomycin, which exhibit potent antimicrobial activity against fungi, bacteria, and γ-proteobacteria, but little activity against other types of microorganisms, are activated in *Drosophila*[101,102].

*Drosophila* synthesize molecules like Spaetzle, that are similar to mammalian cytokines, to orchestrate intercellular communication and to regulate immune cell behaviour and differentiation in response to microbial stimuli[95,96,99].

An overview of the three fundamental networks of signaling for immunity in *Drosophila*, notably the IMD, TLR, and challenges from fungi and Gram-positive bacteria, activate the Toll pathway, which results in the nuclear translocation of the dorsal relish transcription factor. This can enhance the antimicrobial defenses by upregulating the expression of AMP genes[103–105]. Dorsal-ventral patterning during embryonic development is a significant component of the Toll pathway[87]. On the other hand, the IMD pathway utilizes *Relish*, a transcription factor triggered by Gram-negative infections by bacteria, plus other immune effectors that promote the process of bacterial clearance, generating AMPs[87,90,103,106]. Moreover, IMD modulates gut homeostasis and pursues epithelial integrity[89]. The unpaired (Upd) family of proteins notably activates the JAK/STAT pathway, which then, in turn, controls the manufacturing of cytokines and the differentiation of distinguished immune cell types in response to either tissue injury or bacterial infection in *Drosophila*. Furthermore, the insulin homeostasis and immune system functions are influenced by signaling of JAK/STAT pathway[96,107].

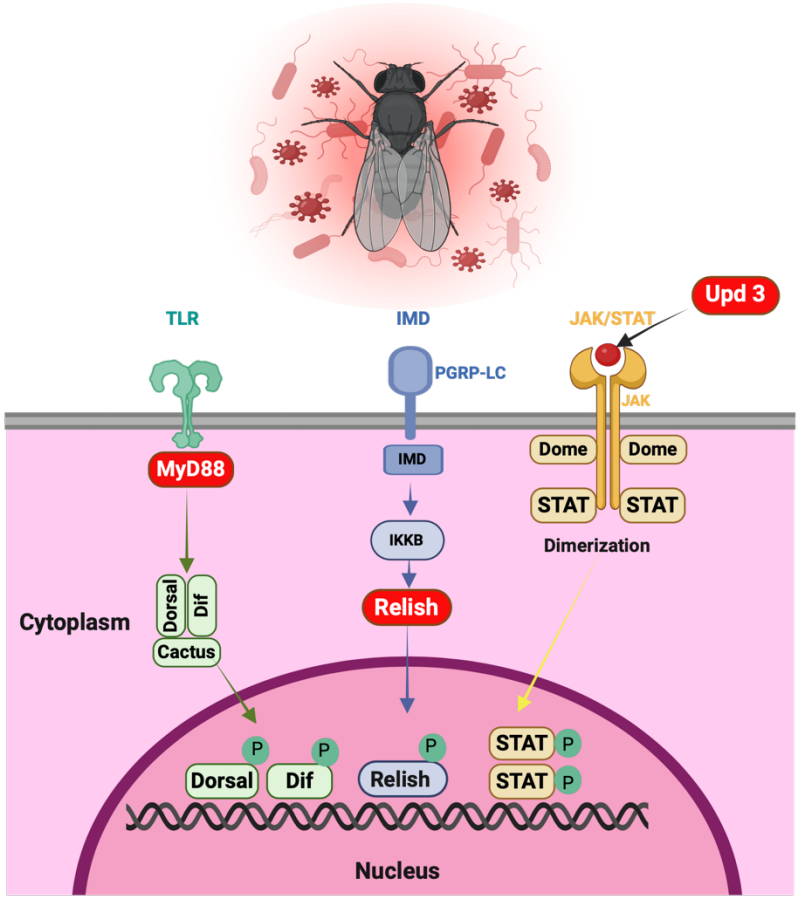



**Figure 3: TLR, IMD and JAK/STAT pathways in *Drosophila melanogaster*.** The illustration represents the cross-talk between these pathways and how the immune system of Drosophila detects pathogens and transduces the signal to initiate the production of antimicrobial peptides and other immune-related genes. The mechanisms deployed by *Drosophila* in host defence against pathogens prominently include Toll, IMD (Immune Deficiency), and JAK/STAT pathways. Further, Cactus sequesters cytoplasmic NF-κB-like transcription factors Dorsal and Dif, following MyD88 activation. They then release themselves from Cactus, locate themselves in the nucleus, and bring about the transcription of antimicrobial peptides. In the IMD pathway, the IKK phosphorylates Relish, an NF-κB-like transcription factor. After the event of phosphorylation, Relish gets into cleavage mode, thereby allowing Relish to enter the nucleus. The following is the transcription of genes involved in the immune response. JAK/STAT Pathway gets activated upon unpaired family members like Upd3. Initially, JAK was activated by Upd3. Sometime after, JAK phosphorylates receptor Domeless and STAT; subsequently, STAT forms a dimer and gets translocated to the nucleus upon activation by JAK. Phosphorylated STAT dimers induce the transcription of target genes involved in immune responses[89,102,104,105,108,109].

Moreover, for the purpose of preserving tissue integrity in immune-mediated homeostasis, the JNK pathway in *Drosophila* is known to control the reaction toward cellular stress and apoptosis, including the process of wound resolution[110]. The JNK pathway promotes both division of cells and morphogenesis through development[111]. Altogether, Drosophila has made it possible to understand the cellular and molecular factors that regulate the signaling and development of the innate immune system. Thus, Drosophila is an efficient model for the investigation of the innate immune system and host-pathogen interactions.[112].

### 1.5.2 Plasmatocytes: The *Drosophila* macrophages

Macrophages have been observed in many tissues, and all circulating and resident macrophages with biochemical features particular to their distinct tissues and embryonic differentiation [37,113]. Plasmatocytes are the fundamental immune cells in clearance of pathogens and maintenance of homeostasis in *Drosophila*[94,114]. Further, it has been shown that plasmatocytes could be having an impact on the overall health of the brain and may be responsible for a potentially dangerous drop in longevity and senescence behaviours[84,96,115]. Likewise, it has been proven that the *Drosophila* cytokines Upd2 and Upd3 are essential for activating the innate immune response, and their productions have been connected insulin and glucose homeostasis which are important in development of obesity[95,96]. These cytokines could impact systemic growth and metabolism through cytokine-mediated pathways employing the secretion of insulin[95,96].

To conclude, *Drosophila* macrophages and plasmatocytes can be modified by maternal nutrition and can lead to the development of vast disorders in the progeny. Moreover, *Drosophila* has similarities to mammals in many organ and physiological functions and preserves components of the immune system such as MyD88, NF-kB and JAK/STAT. Furthermore, the short lifespan of *Drosophila* and its suitability for genetic modifications make it a useful model and are vital for generational studies on maternal obesity. As a result, it is a suitable model for molecular and pathophysiological demonstration of the possible effects of maternal obesity on health and the development of new therapeutic strategies.

### 1.3 Aim of the thesis

This thesis investigates how maternal diet influences long-term health outcomes in offspring, specifically examining the role of the innate immune system in driving these effects. Using *Drosophila melanogaster* as a model, which offers powerful genetic tools and conserved metabolic and immune pathways, the research tests the hypothesis that maternal obesity negatively impacts progeny health and lifespan, with innate immune involvement.

The first objective was to establish an obesity-like phenotype in *Drosophila* by feeding females a high-fat diet (HFD) and a high-sugar diet (HSD). Induction of the obesity-like phenotype was confirmed by measuring plasmatocyte density, glucose levels, lipid profiles, and reproductive output. The second objective evaluated the impact of maternal obesity on offspring health, focusing on longevity, neurodegeneration, and metabolic function, including climbing ability, lifespan, axonal integrity, and metabolic variables in adult offspring at days 0, 10, and 50. The third objective explored whether the innate immune system, particularly plasmatocytes, contributes to the observed pathologies. To assess immune involvement, experiments included bacterial challenges, antibiotic treatments, and the genetic depletion of immune mediators in plasmatocytes, such as Relish, Upd3, and MyD88 (Figure 4).

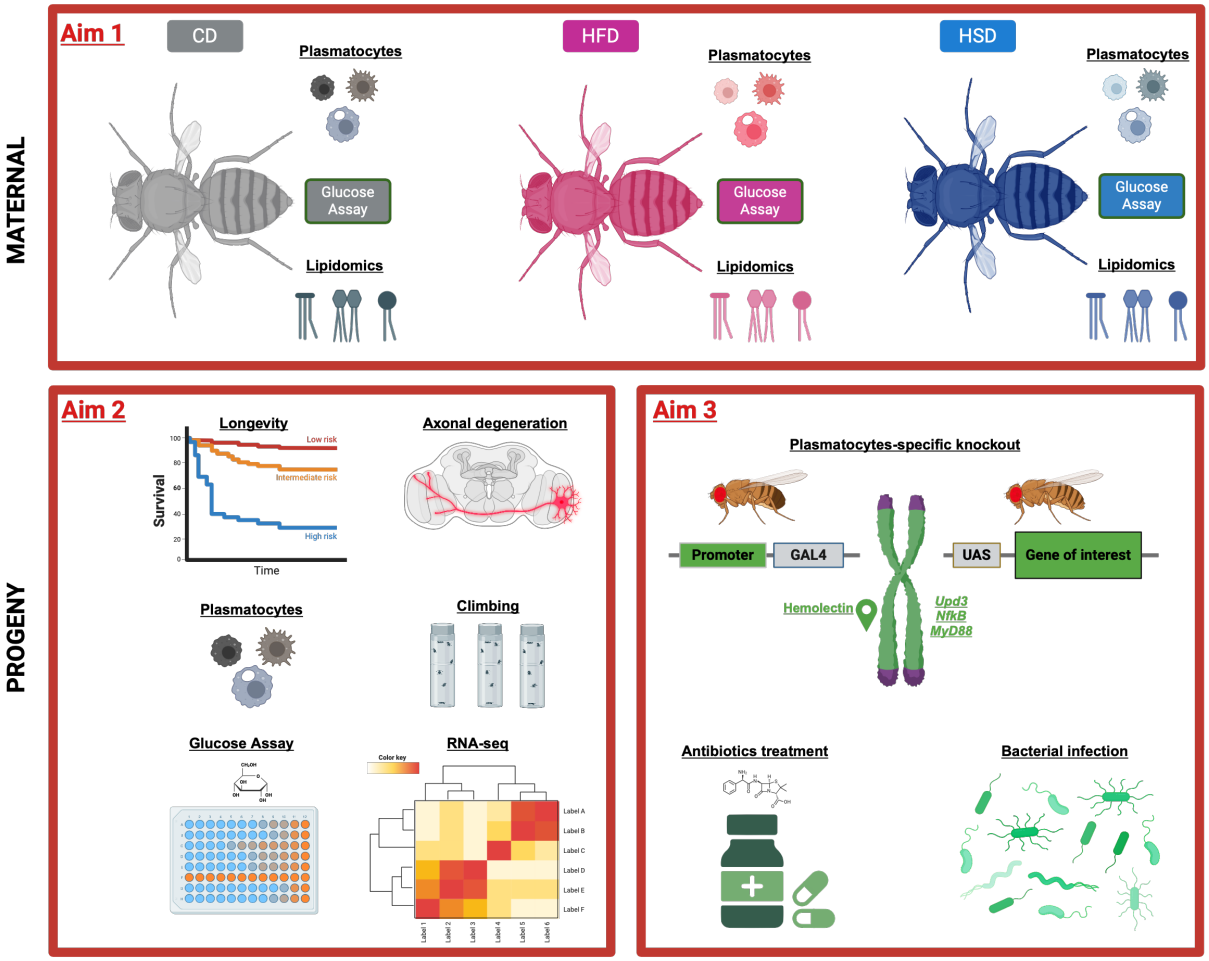



**Figure 4: Aims of the thesis.** The figure illustrates the aims and proposed techniques of the thesis. GAL4: Gal4 transcription factor, UAS: upstream activating sequence, Upd3: unpaired3, NfKB: nuclear factor kappa b, MyD88: myeloid differentiation primary response 8, RNA-seq: RNA sequencing (figure created using the biorender.com).

# Chapter 2: Materials and Methods

## 2.1 Materials

### 2.1.1 General laboratory equipment

**Table 1 Laboratory equipment**

| Material | Model | Company |
|---|---|---|
| Confocal Microscope | LSM 880 | Zeiss |
| Light microscope | AX 70 | Olympus |
| Light microscope | Leica M80 | Leica |
| Centrifuge | Universal 16A | Hettich |
| Fly incubator | | RuMed |
| Spectrometer | Q Exactive Plus | |
| Bacterial incubator | | |
| Microinjector | FemtoJet | Eppendorf |
| Vortexer | | Vortex Genie2 |
| Microscope | Leica M80 | Leica |

### 2.1.2 Consumables

**Table 2 Consumables**

| Material | Company | Catalog # |
|---|---|---|
| Food cages, ø x h = 28.5 x 95 mm | Dominique Dutscher | 789009 |
| Transfer pipettes | VWR | 612-4504 |
| TC-Platte 6 Well Suspension | Sarstedt | 83-3929.500 |
| Petri dishes | VWR | |
| Pipettes | Eppendorf | |
| Brush | | |
| Micro-dissection forceps | Sigma-Aldrich | F3767 |
| Superfrost glass slides | Thermo Fisher Scientific | |

## 2.1.3 Chemicals, reagents and solutions

**Table 3 Consumables**

| Material | Company | Catalog # |
|---|---|---|
| Penicillin-Streptomycin | PAN-Biotech | P06-07100 |
| Nutri-Fly BF | Genesee Scientific | 66-112 |
| Methyl-4-hydroxybenzoate | Sigma-Aldrich | 99-76-3 |
| Propionsäure (Propionic Acid) | Sigma-Aldrich | 79-09-4 |
| Nipagin (Methyl Paraben) | Sigma-Aldrich | 99-76-3 |
| Water | | |
| Qiazol | Qiagen | 79306 |
| RNeasy UCP Micro Kit (50) | Qiagen | 73934 |
| Chloroform for analysis EMSURE® ACS, ISO | Merck | 1024451000 |
| RNase AWAY™ Surface Decontaminant, Marke: Thermo Scientific™ 7000 | Fisher Scientific | 11580095 |
| Ethanol 100 % | AppliChem | A3678,1000 |
| High Sensitivity RNA Kit | Agilent Technologies | 5067-5580<br>5067-5579<br>5067-5581 |
| Chloroform LC-MS grade | Merck Millipore | 1024421000 |
| Isopropanol LC-MS grade | Merck Millipore | 1027811000 |
| Methanol | Merck Millipore | 1060352500 |
| 1x PBS (w/o MgCl2 and CaCl2) | | |
| mouse anti-Chaoptin | DSHB | 24b10 |
| Anti-mouse Alexa 594 | Life Technologies | |
| Glucose Oxidase/Peroxidase Reagent (GO) | Sigma-Aldrich | G3660 |
| o-Dianisidine Reagent | Sigma-Aldrich | D2679 |
| Assay Reagent | Sigma-Aldrich | |
| Glucose Standard Solution | Sigma-Aldrich | G3285 |
| Glue | Pritt | all-purpose glue |

## 2.2 Experimental design

### 2.2.1 Fly strains, husbandry and induction of maternal obesity

#### *2.2.1.1 Fly strains*

**Table 4 Fly strains**

| Genotype | Annotation | Source | Stock # |
|---|---|---|---|
| $W^{1118}$ | White mutant | Bloomington | 6326 |
| y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00646}attP2 | UAS-Upd3.dsRNA | Bloomington | 32859 |
| w1118;HmlΔ -Gal4,UAS-2xeGFP | Hml-GAL4 | gift from Katrin Kierdorf | N/A |
| w[1118]; P{w[+mC]=10XStat92E-GFP}2 | Stat92E-GFP | Bloomington | 26198 |
| GMRwhite RNAi/GMRwhite RNAi;GMR-gal4/GMR-gal4;UAS-tubGFP/MKRS | Orange mutant | gift from AG Tavosanis | N/A |
| y[1] v[1]; P{y[+t7.7] v[+t1.8]=U6.2-Myd88.dgRNA}attP2 | MyD88.dgRNA | Bloomington | 94297 |
| y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00070}attP2 | UAS-rel.dsRNA | Bloomington | 33661 |

In order to obtain hemocyte-specific knockdown, males bearing the UAS-rel.dsRNA (33661), UAS-MyD88.dgRNA (94297), or UAS-Upd3.dsRNA (32859) transgenes were mated with virgin females harbouring the Hml-GAL4 (30140) transgene. To obtain the genotypes of GMRwhite RNAi/white, GMR-gal4/+, and UAS-tubGFP/+, virgin females of the orange mutant bred with males of the white mutant for axonal degeneration examinations.

#### *2.2.1.2 Husbandry*

Whole maintenance groups and the progeny that emerged from each group were raised in a climate-controlled incubator sustained at 25°C, with persistent humidity and light and dark cycles. They were fed solely with CD food.

#### *2.2.1.3 Induction of maternal obesity*

To induce maternal obesity, the experimental diets were prepared based on a commercially available food substrate, Nutri-fly, which enriched with essential nutrients such as yellow cornmeal, soy flour, agar, inactive nutritional yeast, corn syrup. The quantity of food was calculated based on an estimation of 1 ml containing 10 ml of 10%

nipagin for preservation and 5 ml of propionic acid per litre of food solution. For the HFD, 20% coconut oil, based on dry weight of the food, for HSD 20% sucrose included to CD diet, based on total volume.

The experimental setup included exposure of 2-5 days female flies to 7 days of HFD, HSD, or a control diet of CD. Following, the females of every group were mated with CD males in a CD cage after these had completed 7 days of duration. The progeny was raised with CD in all cases. All progeny were placed on CD food and raised in a 25°C incubator with a light-dark cycle. The progeny was measured in several stages: day 0, day 10, and 50 (Figure 5).

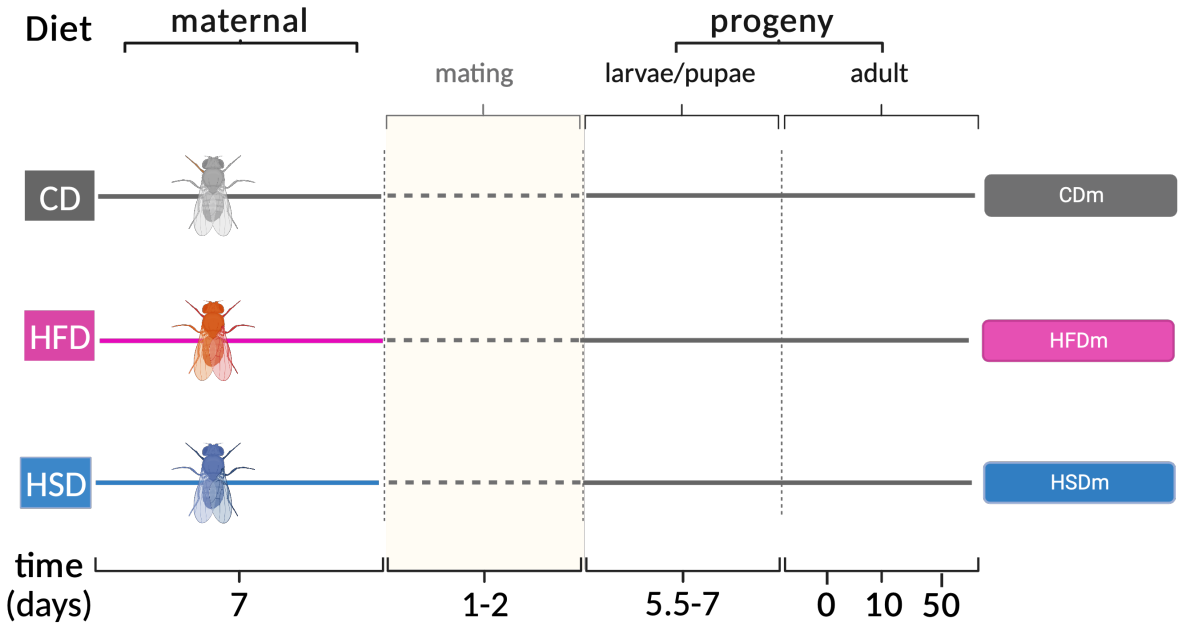



**Figure 5: Experimental design of maternal obesity in *Drosophila*.** The figure demonstrates the experimental design of the research. The female flies were fed with CS, HFD or HSD for 7 days, and all groups of females mated with CD males on CD food. CD: Control diet, HFD: High-fat diet, HSD: High-sugar diet, CDm: Progeny of control diet-fed flies, HFD: Progeny of high-fat diet-fed flies, HSD: Progeny of high-sugar diet fed mothers

## 2.3 Metabolic and phenotypic assays

### 2.3.1 Measurement of body weight

Single flies were lined carefully on a sterile petri dish over ice to secure a constant and, more considerably, controlled environment for the experiment. Their individual body weight was measured using a precision scale.

### 2.3.2 Measurement of circulating glucose level

The assay reagent preparation weight of 39.2 ml of the Glucose Oxidase/Peroxidase Reagent (GO), (Sigma-Aldrich, G3660) was taken and appropriately mixed with 0.8 ml of the o-dianisidine reagent which supplied inside the kit.

Following proper handling procedures, flies were lysed in 100 µl of PBS on ice to prevent enzymatic digestion of glycogen and trehalose. The resultant homogenate was centrifuged at 2000 G for 5 minutes, and the supernatant was boiled at 70 degrees Celsius for 10 minutes.

Samples of the glucose standards 0, 0.02, 0.04, 0.08, and 0.16 milligrams per millilitre were prepared for the glucose standard solution and for making the standard curve.

A clear-bottomed 96-well plate was used for the assay, and first level of wells was filled with 50 µl of every glucose standard, and the rest of the wells were filled with 50 µl of the prepared samples. 50 µl of the assay reagent were pipetted gently in the glucose standards and those containing the samples.

Eventually, samples were incubated at 37°C for precisely 45 minutes for the reactions. The reactions are then stopped by adding 100 µl of 1.8 molar sulfuric acid to wells. Absorbances of the samples in a plate are then measured using a plate reader at 540 nm.

### 2.3.3 Lipidomics

Samples are initially mixed with 200 µl of extraction mix ((CHCl3/MeOH 1/5 containing internal standards: 250 pmol PE (31:1), 472 pmol PC (31:1), 28 pmol CL (56:0), 98 pmol PS (31:1), 84 pmol PI (34:0), 56 pmol PA(31:1), 51 pmol PG (28:0), 39 pmol LPA (17:0), 45 pmol Car (15:0), 35 pmol LPC (17:1), 38 pmol LPE (17:0), 32 pmol Cer (17:0), 240 pmol SM (17:0), 55 pmol GlcCer (12:0), 340 pmol TG (50:1-d4), 111 pmol CE (17:1), 64 pmol DG (31:1), 103 pmol MG(17:1), 724 pmol Chol (d6)), followed by resuspension in another 300 µl of the extraction mix. The resulting mixture was then

ultrasonicated for 30 minutes using a bath sonicator in order to break cellular material and allow effective extraction.

After sonication, the samples were centrifuged with a force of 20,000×g for 2 min, which helped in the separation of cellular debris and other particulate material that may be present. The supernatant, rich in the desired analyte, was carefully transferred into a new Eppendorf to avoid contamination.

200 µL chloroform and 800 µL of 1% acetic acid solution in water are gently homogenized to refine the sample preparation process further. The mixture was then manually agitated for 5 seconds to set the phase separation and re-centrifuged at 20,000 times gravitational acceleration for 2 min to allow the isolation of the lower phase, which contains the target analytes of interest.

The lower phase was then transferred into the fresh Eppendorf tube and evaporated in speed vac at 45°C for 15 minutes. This step allowed saturation, concentrating the charge analytes to be further analyzed. Finally, after the concentration process, the samples were made homogeneous with 1000µL of spray buffer using an ultrasonic bath for 5 minutes to properly distribute the analytes in the homogeneous solvent matrix. For shotgun lipidomics, each sample was individually measured at a rate of 10 µl/min into a Thermo Q Exactive Plus spectrometer that was equipped with a HESI II ion source. Positive mode MS1 spectra with a resolution of 280 000 were captured in 100 m/z windows ranging from 250 to 1200 m/z and subsequently by recording MS/MS spectra (resolution 70 000) by data-independent acquisition in 1 m/z windows from 250 to 1200 (positive mode). Using custom mfql files, raw files were converted to mzml files and then loaded into LipidXplorer software, which was used to analyse and identify sample lipids and internal standards. The internal standard intensities were used to calculate absolute amounts for additional data processing. Raw data were converted to picomol (pmol), and the mean value of all peaks was obtained based on pmol values for each lipid class. The quantification of the extracted lipids, normalized by the body weights of individual flies.

## 2.4 Climbing assay

### 2.4.1 Experimental setup

After hatching, the flies were sorted into cages, each having 20–25 flies according to sex and age. The flies were then pursued under normal conditions and allowed to feed on a CD diet until day 10 and day 50–the experimental days. One day before the assay, the flies were recounted and transferred into fresh cages. On the day of experimentation, flies were transferred to climbing assay tubes and video documented for their climbing ability.

### 2.4.2 Quantification

The images were imported into Fiji and saved. The photographs were scaled with Fiji, where the straight-drawing tool was used to measure a fixed distance of 10 cm on the millimetre paper in the Fiji toolbar.

With the help of the angle tool in the Fiji toolbar interface, the 10 cm mark was selected to draw a rectangle from, and the climbing dimension was noted for each fly with the shortcut command noted as "strg+M". Flies not observed with a hold on at the bottom were noted with a climbing height of zero.

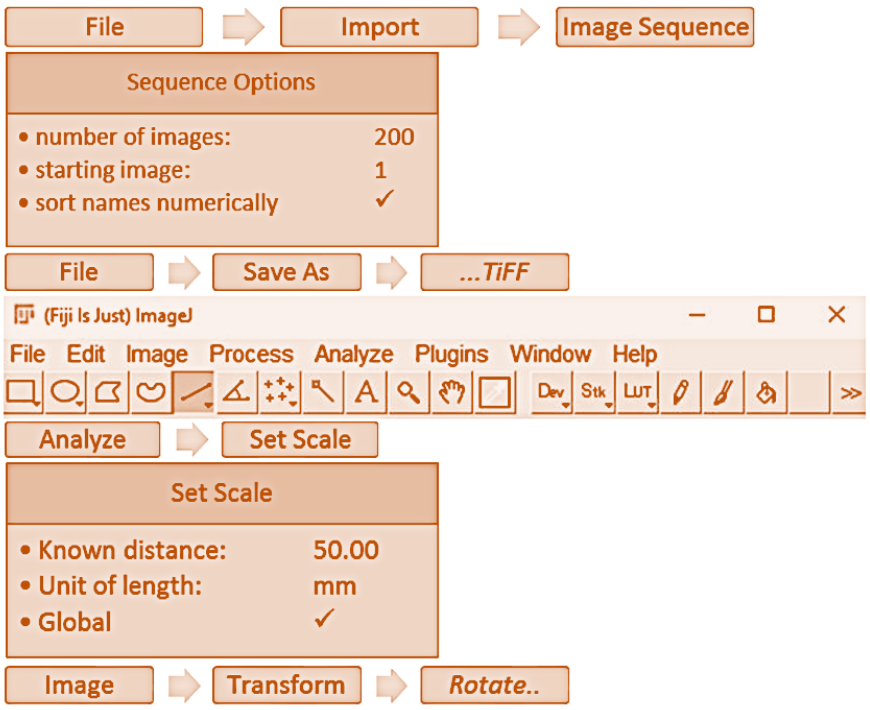



**Figure 6: Quantification of the climbing performance using the Fiji software**

Following, counts were plotted in GraphPad Prism, and data were viewed in the results window where they appeared in the "height" column. All measurements were made on a single image stack (column "Slice"). Next, for measurements on the line drawn from the 10 cm reference, the height was adjusted for flies below this line as 100 less than the "height" measured. The height was adjusted for those above the line to be 100 plus the "height" measured.

### 2.5 Assessment of longevity

For the longevity assay, usually 10-16 flies were gently transferred into a new cage, and the numbers of each replicate were tried to be kept equal for each genotype depending on the age of the flies.The flies were transferred into new vials and cages at least two times each week after eclosion. Each experimental replicate had a minimum of three maternal groups per genotype. The whole experimental population was kept at 25° C. Lifespan counting was determined by counting the number of deaths during the experiment'.

### 2.6 Axonal degeneration

#### 2.6.1 Experimental design

Males and females of a correct genotype were selected and kept to mature on CD for 10 days under standard conditions. Thereafter, mature adult females and males were placed on CD into individual vials containing up to 10 animals. Next, one vial of females and males from CD, HSD, and HFD parental exposure was kept under standard conditions for 7 days in LD, 25°C, and 60% humidity, which served as a control group. The second vial of females and males from CD, HSD and HFD parental treatment were exposed to continuous light conditions for 7 days (LL, 25°C and 60% humidity). As previously stated in Richard et al., 2022[116], an LED light source was employed (6W, warm white, Heitronic), and the flies were transferred food vials at an illumination level of 10,000 lux. The intensity was determined at the peak of the food in the vials and along the path of the light source (Volt Craft MS-1300 photometer, Conrad) [116]. Briefly, Control flies (LD) and flies exposed to light (LL) were further immobilised on ice, followed by adult brain preparation performed in RT 1X PBS, and brains were fixed in cold 4% formaldehyde for 50 min at RT. Samples were subsequently washed with PBS containing 0.3% Triton X-100. Subsequently, brains were stained with mouse anti-

Chaoptin (DSHB, 24b10) primary antibody (1:25) and incubated overnight at 4°C in PBS containing 0.1% Triton X-100 and 1% BSA. Anti-mouse Alexa 594 (Life Technologies) in 1:400 dilution was used as a secondary antibody (RT, 2-3h). Then, brains were mounted in their posterior orientation in Vectashield (Vector Laboratories). To preserve the brain's original shape and volume, 0.1 mm insect pins were used (Entomoravia). All experiments were executed in AG Tavosanis with the help of Karolina Doubková.

### 2.6.2 Quantification

Standard confocal microscopy using Zeiss LSM 780 was performed to visualise axonal termini in the medulla. A z-stack of full medullas was collected using a 40x oil objective with the following settings: fixed interval 1um, 1AU, 1024 frame size, 8-bit image type). Images were processed using Fiji software (Schindelin et al., 2012), and 3D reconstruction and semi-automated quantification of axonal termini were performed using Imaris software (Bitplane, 9.7.2. software). A compact 3D mask of R7 termini was created by automated software developed by Nitta et al., 2023. Subsequently, manually counted R7 axonal terminals as described in Richard, Doubkova et al., 2022 using previously determined settings to produce the most reliable counts of R7 terminals[116]. Data was further imported into GraphPad Prism software to provide accurate statistical analysis of obtained data.

## 2.7 Count of plasmatocytes

### 2.7.1 Microscopy settings

Flies expressing hemolectin-specific green fluorescent protein (hml-GFP) were utilized, and they were subsequently immobilized onto slides using a novel lateral fixation method employing adhesive glue. This approach fulfils a distinct means of studying the localization and dynamics of hemocytes within *Drosophila melanogaster.*

The imaging process involved using Zeiss LSM 880 Airyscan confocal microscopy with fluorescence excitation set at 488 nm in 10X objective. In order to adjust image quality, the gain, pinhole, intervals, and tile scan arranged to 757, 1 AU, 25 μm 4x3, respectively.

2.7.2 Quantification

In order to analysis and quantify the plasmatocytes count, the image stacks were transformed into maximum intensity projections and converted to tiff format in Fiji software. Afterwards, hemocytes were quantified utilizing a 3D object counter with a threshold of 220-240, ensuring robust detection while minimizing background noise.

## 2.8 Assessment of the JAK/STAT pathway activation

2.8.1 Microscopy settings

w[1118]; P{w[+mC]=10XStat92E-GFP}2 Stat92E-GFP, flies expressing GFP in JAK/STAT were fixed in slides using a lateraly using adhesive glue.

The Zeiss LSM 880 Airyscan confocal microscopy was used for imaging, with fluorescence excitation set at 488 nm at 10X magnification. The gain was set to 818, pinhole size to 1 AU, intervals to 25 µm, and a 4x3 tile scan configuration was used.

2.8.1 Quantification

In order to determine the JAK-STAT activity in *Drosophila*, to have tiff format pictures z-stacks in max intensity transformed to 3D. Subsequently, the GFP signal was quantified using all fly bodies' mean fluorescence intensity (MFI).

## 2.9 Bulk-RNA sequencing

2.9.1 Isolation

Before starting, all equipment and surfaces were cleaned well with RNase-free water. Samples were run at room temperature unless stated otherwise. The samples were thawed and homogenized with the Precellys®24 (peqlab) system at 5000, 15 seconds homogenization. Subsequently, the samples were centrifuged at 13,000 g for 30 s and the resulting supernatant was pipetted carefully into a new tube to remove the residual glass beads.

The homogenized samples were mixed with chloroform in a 1:5 ratio of chloroform to Qiazol for RNA extraction. After incubation, briefly centrifuge at 13,000 g for 15 min at 4°C. Transfer the upper aqueous phase enriched with RNA to a fresh tube; add 300 µl of 100% absolute ethanol immediately.

Next, the prepared RNA-containing solution was pipetted into a 2 ml RNeasy MinElute spin column and centrifuged at 8000 g for 15 seconds. After discarding the flow-through, 80 µl of DNase buffer per sample was added to the remaining sample, followed by a 15-minute incubation.

Further, 350 µl of RW1 buffer was added to the spin column, followed by centrifugation at 8000 g for 15 seconds, and the supernatant was discarded, all of which tempts me to take out the spin column and transfer it to another 2 ml collection tube. The spin column was washed with 500 µl of Buffer RPE and centrifuged at 8000 g for 15 seconds. Following this step, 500 µl of 80% ethanol was pipetted into the spin column, and the latter was centrifuged at 8000 g for 15 seconds. The flowthrough and collection tubes were disposed of.

Finally, the elution of the purified RNA was done by picking the spin column from the new 2 ml Collection Tube and centrifuging for 5 minutes at 13,000 g. Afterwards, the spin column was very cautiously pipetted in a new 1.5 ml tube, followed by direct pipetting of 14 µl of RNase-free water into the middle of the spin column membrane. After centrifugation for 1 min under 13,000 g, the samples eluted in RNA were promptly stored in a deep freezer at -80°C until further use.

### 2.9.2 Library preparation and sequencing

Quality control was performed by employing the Agilent RNA 6000 pico Kit (Agilent Technologies, #5067-1513) and the Agilent 2100 Bioanalyzer; the RNA integrity number (RIN) was determined following the manufacturer's instructions to assess the quality of the RNA and estimate the degree of degradation. Alignments were made in the core facility of the European Molecular Biology Laboratory in the Heidelberg using the STAR aligner (version 2.7.9a) to align the fastq files on the dm6 genome with default parameters.

### 2.9.3 Quantification and analysis

The acquired reads were aligned and quantified using Kallisto[117]. Initially, an index was constructed using the transcriptome FASTA files of the organism, after which quantification was done using fast files. After unidentified transcripts were eliminated, h5 files were read into R (v. 4.2.0) and integrated with the labelling from **.gtf for further

gene symbol and biotype annotation. To create a summarizedExperiment[118] object, the tximport (v. 1.24.0)[119] and DESeq2 (v. 1.36.0)[120] packages were employed.

A final gene collection of 12,631 was obtained via preprocessing, which included keeping only protein-coding genes and eliminating continuously expressed genes that had fewer than ten counts in more than 25% of the data. Variance-stabilized counts were employed for principal component analysis (PCA). Male samples M8 and female samples F12 were eliminated because they clustered with the opposite sex, most likely as a result of processing errors. The dataset was first divided into gender-specific subsets, and the previously indicated data processing procedures were then applied to the smaller sets. This resulted in a final gene count of 12,614 for the male and 10,481 for the female set. Differential expression analysis was conducted using DESeq2, using a strategy that considered the impact of interaction between the two main components (Diet and Day). Respective contrasts were specified within the DESeq2 results function. A gene was deemed significant if p. adj < 0.1, with LFC > 0 indicating upregulation and LFC < 0 indicating downregulation.

### 2.10 Antibiotics treatment

Antibiotics-integrated CD food was prepared by measuring 90 g of Nutri-Fly BF and homogeneously mixing it with 508,5 ml distilled water until a homogenous mix was achieved. The mixture was then heated slightly in high option (800-1000W) of the microwave so that food pellets would not form. The mixture was cooled until it was sufficiently safe for handling. 5,11 ml of 10% nipagin, 2,45 ml propionic acid, and 2.5 ml penicillin-streptomycin were added to get approximately (considering the evaporations) 1%, 0.5%, and 0.5% concentrations, respectively, were added. The obtained food cages after preparation were received in the designated food cage at 5-6 ml, dried overnight at room temperature, and the following day placed in the cold room at +4°C. The use of the prepared food cages within 3-4 weeks was a critical aspect, and during all the preparation, high accuracy in measurements was pursued, as well as a strict hierarchy of the aseptic procedures. Proper storage of food additives according to the manufacturer's (Nutri-Fly BF, Genesee Scientific 66-112) recommendations was necessary, and monitoring the state of the prepared product during storage was recommended to respond promptly to signs of contamination.

## 2.11 Bacterial infection

### 2.11.1 Preparation and injection of bacteria

A colony of *E. coli* from each plate kept at 4°C was inoculated into 10 ml of LB media, supplemented with 100 µg/ml ampicillin, and cultured overnight at 37°C. The density of the bacterial culture was determined on the day of the experiment by taking an optical density (OD600) reading at 600 nm using LB medium as a blank, with the culture diluted to give an OD600 value of 1 using LB medium. To achieve the desired number of bacteria per injection, the bacteria were centrifuged and resuspended using 100 µl of PBS. Injections were applied in 25 nanoliter (nl) volumes using an Eppendorf Femtojet microinjector with the help of Lennart Gollenbeck from AG Schmuker.

### 2.11.2 Survival assay

To illuminate the complex dynamics of survival post-infection, three sets of 15 flies per condition were investigated after the bacterial or PBS injections. The flies were raised in standard humidity, and dead flies were counted daily.

### 2.11.11 Bacterial load

Infected *Drosophila* was submitted to $CO_2$ for anaesthetizing, gently placed on a sterile surface, and quickly transferred to ice to keep the sample intact. The flies were washed with 70% ethanol using the shaker for 30 s, followed by academia rinsing with PBS for one minute. Flies were placed in separate microcentrifuge tubes, and 500 µl sterile PBS were added to individual tubes with sterile forceps. Homogenization of the samples was carried out in 3 cycles, with a programmed setting of the Precellys®24 (peqlab) system at 5000, 15 seconds homogenization, and a pause of 20 seconds between cycles. The samples were then centrifuged post-homogenization, and a series of separate dilutions, $10^{-1}$, $10^{-2}$, $10^{-3}$, and $10^{-4}$, were made in microcentrifuge tubes using sterile PBS. To ensure proper dispersion, each dilution was properly vortexed. The sterile LB agar plates will be labelled with appropriate sample IDs and dilution factors. Spot plating will be done, spotting exact volumes from each dilution onto the agar plates. The plates will be spread out to distribute the bacterial suspension across the agar surface. The plates are following incubated at 37°C for 24 hours upside down so that the air will dry out. Next, the bacterial colonies on each plate were

counted, and the number of colonies was recorded for each group. CFU per fly was calculated by the average quantity of colonies, dilution factor, total volume, plated volume, and the number of flies in each sample.

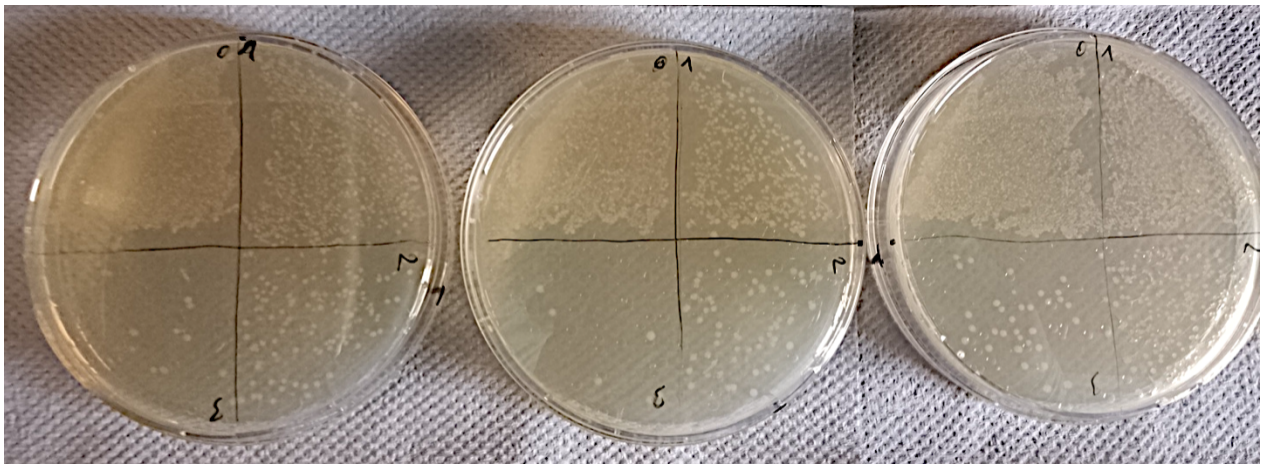


**Figure 7: The colony forming unit assay.** For the colony-forming unit (CFU) assay, the bacterial density of the samples was measured at various concentrations to determine the countable bacterial population at various bacterial concentrations. These measurements were taken on day 0 and subsequently on the following days to monitor changes in bacterial density over time.

## 2.13 Statistical analysis

Statistical analyses were examined using GraphPad Prism 10. Comparisons for most of the group differences were conducted by one-way ANOVA. Survival assays were analyzed using the Wilcoxon test, and levels of statistical significance were indicated as * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and **** for $p < 0.0001$.

# Chapter 3: Results

## 3.1 Effects of HFD and HSD in *Drosophila melanogaster*

HFD and HSD represent a particularly advantageous model for the examination of metabolic disorders such as obesity. In order to examining maternal phenotype, the lipidomics, glucose level, productivity and plasmatocyte density investigated.

### 3.1.1 Assessment of lipid species in females upon exposure to HFD and HSD

The lipidomics of whole flies were investigated to reveal the role of HFD and HSD in the lipid metabolism of females and whether the present approach would lead to a maternal obesity model. Lipidomics of HFD and HSD-fed flies demonstrated that both diets raised the lipid content of female flies after 7 days and induced metabolism. A heat-map-based statistical analysis was performed for visualization and interpretation (Figure 8). This analysis unravelled different lipid species upregulated in response to these diets. On the one hand, increased levels of diacylglycerols (DAG), DAGs containing incorporated isomers (DAGIS), and lysophosphatidylethanolamine (LPE) increased in response to HFD and HSD were observed. On the other hand, Triacylglycerols (TAG) were increased in the HSD group, while lysophosphatidylcholine (LPC) in its hydroxylated form (LPCH) was increased in the HFD group (Figure 8).

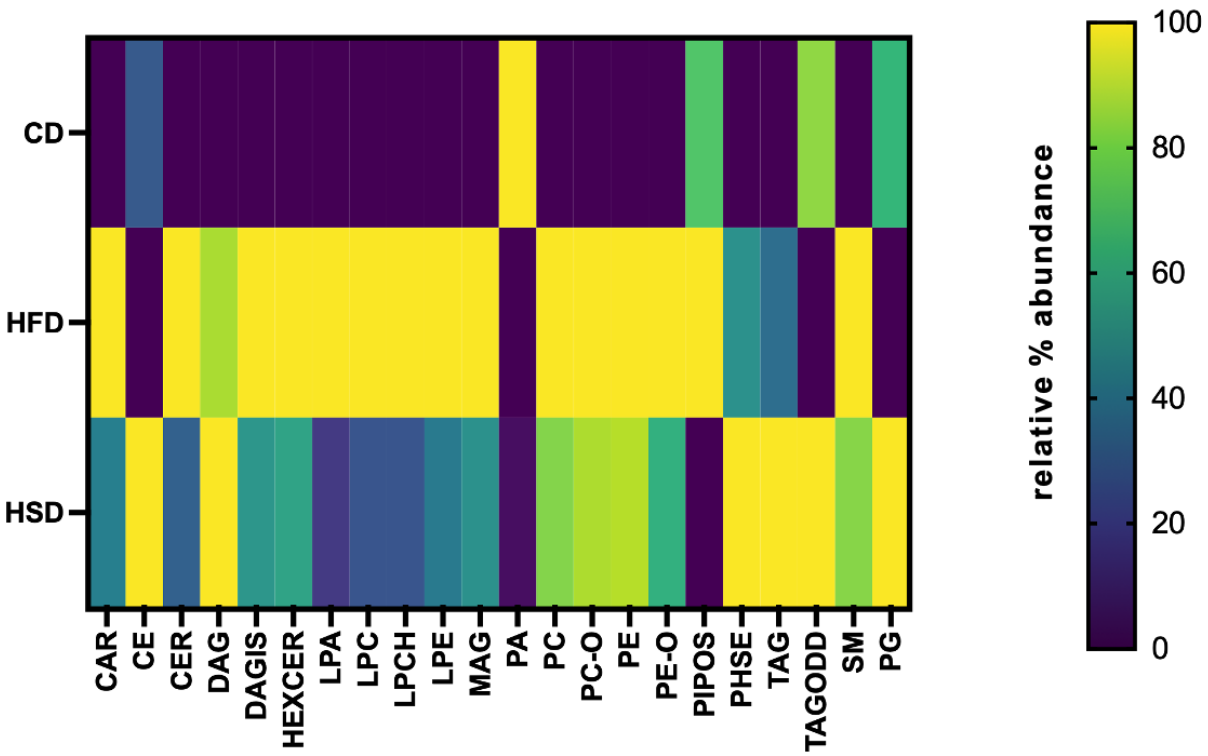



**Figure 8: Heatmap of Drosophila's lipidome.** The figure illustrates the lipidome of mother flies after 7 days of exposure to particular diets. CD: Control diet, HFD: High-fat diet, HSD: High-sugar diet, CAR: Cardiolipin, CE: Cholesteryl Ester, CER: Ceramide, DAG: Diacylglycerol, DAGIS: Diacylglycerol Isomer, HEXCER: Hexosylceramide, LPA: Lysophosphatidic Acid, LPC: Lysophosphatidylcholine: LPCH: Lysophosphatidylcholine Hydroxy, LPE: Lysophosphatidylethanolamine, MAG: Monoacylglycerol, PA: Phosphatidic Acid, PC: Phosphatidylcholine, PC-O: Plasmalogen Phosphatidylcholine, PE: Phosphatidylethanolamine, PE-O: Plasmalogen Phosphatidylethanolamine, PIPOS: Phosphatidylinositol Polyphosphate, PHSE: Phosphatidylserine, TAG: Triacylglycerol, TAGODD: Odd-Chain Triacylglycerol, SM: Sphingomyelin, PG: Phosphatidylglycerol. The bar scale indicates relative % abundance in the particular lipid class.

Furthermore, specific types of lipids, such as carnitines (CAR) and some sphingolipids, like ceramides (CER) and hexoceramides (HEXCER), phosphatidylethanolamine (PE), and plasmalogen phosphatidylethanolamine (PE-O) and were only increased in the HFD group, while cholesteryl esters (CE) were increased in the HSD group. Notably, phosphatidylglycerol (PG-M) was significantly lower in the HFD compared with control and HSD, suggesting that in the instance of fat-rich diets, there was a purse lipid remodelling process (Figure 9).

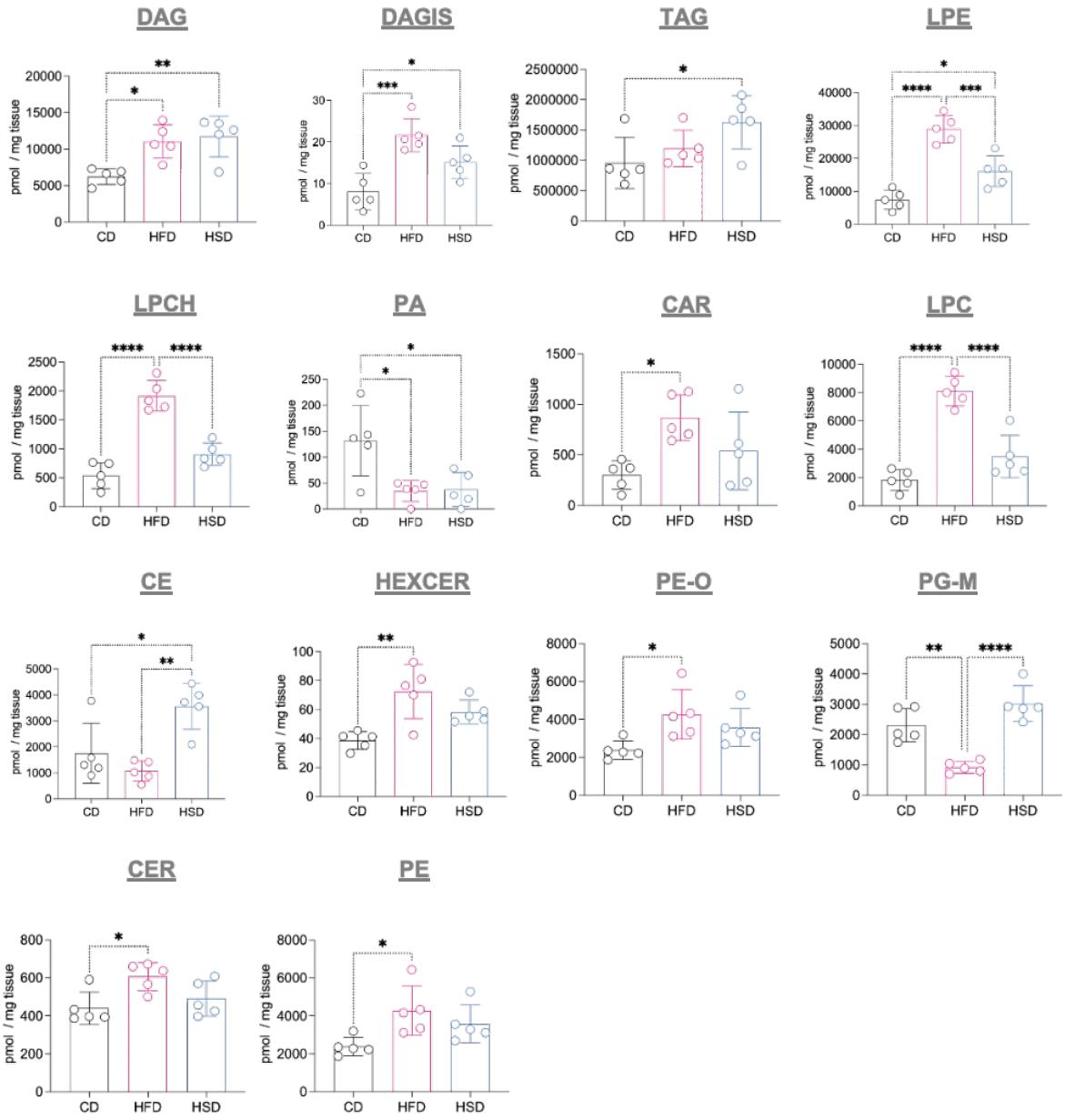



**Figure 9: Lipidome following 7 days of HFD and HSD.** Bar graphs show the absolute values of lipid species in female flies. CD: Control diet, HFD: High-fat diet, HSD: High-sugar diet, DAG: Diacylglycerol, DAGIS: Diacylglycerol Isomer, LPC: Lysophosphatidylcholine: LPCH: Lysophosphatidylcholine Hydroxy, LPE: Lysophosphatidylethanolamine, PA: Phosphatidic Acid, CER: Ceramide, CE: Cholesteryl Ester, TAG: Triacylglycerol, PE: Phosphatidylethanolamine, CAR: Cardiolipin, PG: Phosphatidylglycerol, plasmalogen phosphatidylethanolamine (PE-O). Statistical differences were tested by one-way ANOVA using GraphPad Prism 10. Statistical significance levels were as follows: * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and **** for $p < 0.0001$ (n=5 for all samples).

Although every species exhibited alterations, there was no significant presence in the any dietary groups for lysophosphatidic acid, monoacylglycerol, plasmalogens, phosphatidylserine ethers, phosphatidylinositol phosphates, and sphingomyelins (Figure 10).

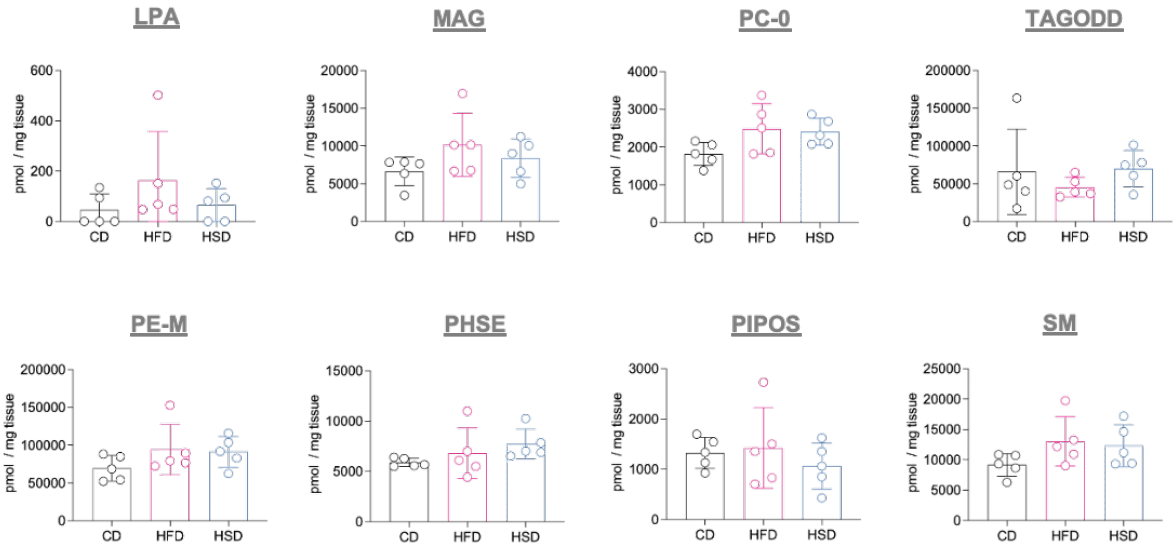



**Figure 10: Lipid species that remained steady.** Bar graphs indicate lipid species that remained steady for the mothers. CD: Control diet, HFD: High-fat diet, HSD: High-sugar diet, LPA: Lysophosphatidic Acid, MAG: Monoacylglycerol, PC: Phosphatidylcholine, PC-O: Plasmalogen Phosphatidylcholine, PIPOS: Phosphatidylinositol Polyphosphate, PHSE: Phosphatidylserine, SM: Sphingomyelin. Statistical differences were tested by one-way ANOVA using GraphPad Prism 10. Statistical significance levels were as follows: * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and **** for $p < 0.0001$ (n=5 for all samples).

### 3.1.2 The role of the diet on the physiology and systemic metabolism of females

In order to shed light on metabolic and physical changes using calorimetric glucose tests, the glucose levels of female flies fed 7 days of HFD or HSD were investigated. Both HFD and HSD had glucose levels comparable to CD. Subsequently, the body weight of flies measured and unlikely human obesity flies in HFD and HSD groups declined body weights drastically compared to CD. As obesity could decline productivity, the pupae density was investigated per fly, and only the HFD group declined productivity compared to CD and HSD. Moreover, the cell numbers were quantified to investigate whether diet sources influence the number of plasmatocytes, the primary immune cells of flies. There was no statistical difference between groups regarding the count of plasmatocytes (Figure 11).

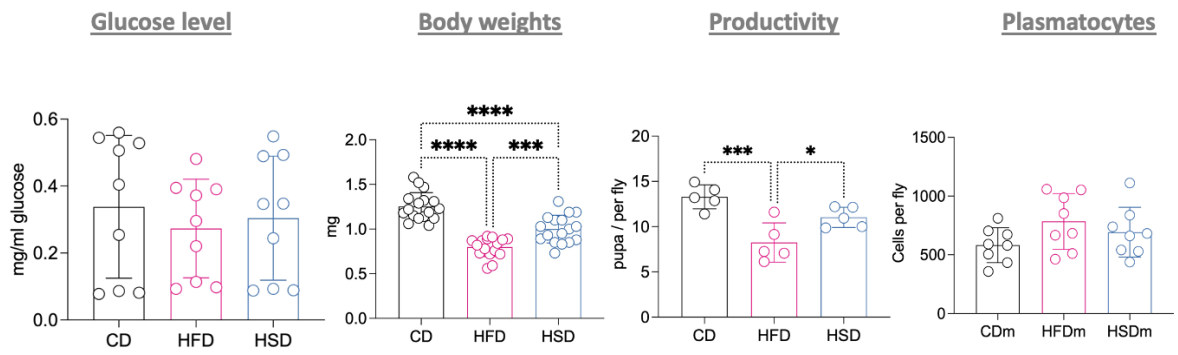



**Figure 11: Glucose level, body weight, productivity and plasmatocyte counts in females fed with HFD and HSD for 7 days.** Bar graphs illustrate mother flies' glucose level, body weight, productivity and plasmatocyte counts, respectively. CD: Control diet, HFD: High-fat diet, HSD: High-sugar diet. Bar graphs demonstrate the glucose levels, body weights, and count of pupae. Statistical differences were tested by one-way ANOVA using GraphPad Prism 10. Statistical significance levels were as follows: * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and **** for $p < 0.0001$ (n=9 for all samples in the glucose test, n=17 for all samples in the body weights measurements, n=5 for all samples in the productivity test, n=8 for all samples in the plasmatocytes count).

In summary, 7 day consumption of HFD and HSD raised majority of lipid classes and decreased the body weight. Moreover, productivity declined solely in HFD consumed flies, while plasmatocyte counts and glucose levels remain constant in both HFD na HSD diets.

### 3.1.3 Transcriptional changes in mothers fed with HFD and HSD

RNA sequencing and data analysis was conducted to understand the prevalent distinctions in HFD- and HSD-fed mothers compared to CD. The data was batch-corrected and visualized using a principal component analysis (PCA) that recapitulates the expression traits of each instance. For PCA, variance-stabilized counts were used The PCA did not show an apparent partition between dietary groups. Moreover, only few differentially expressed genes (DEGs) were detected (Figure 12-A). 27 genes were downregulated in the HSD group compared to CD, and two genes in the HFD group. There were no shared genes downregulated between the HSD and HFD when compared to CD. Relatively more genes were upregulated (Figure 12-B). 30 genes were upregulated in the HSD group compared to the CD group, and this was limited to 18 genes in the HFD group. Moreover, “FBgn0000715”, “FBgn0010019”, “FBgn0029172” and “FBgn0283499” genes were upregulated in both the HFD and HSD fed flies, respectively (Figure 12-C). Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) were performed to understand which pathways and terms are responsible for the differentially expressed genes. There was no enrichment for upregulated and downregulated genes in HFD and HSD.

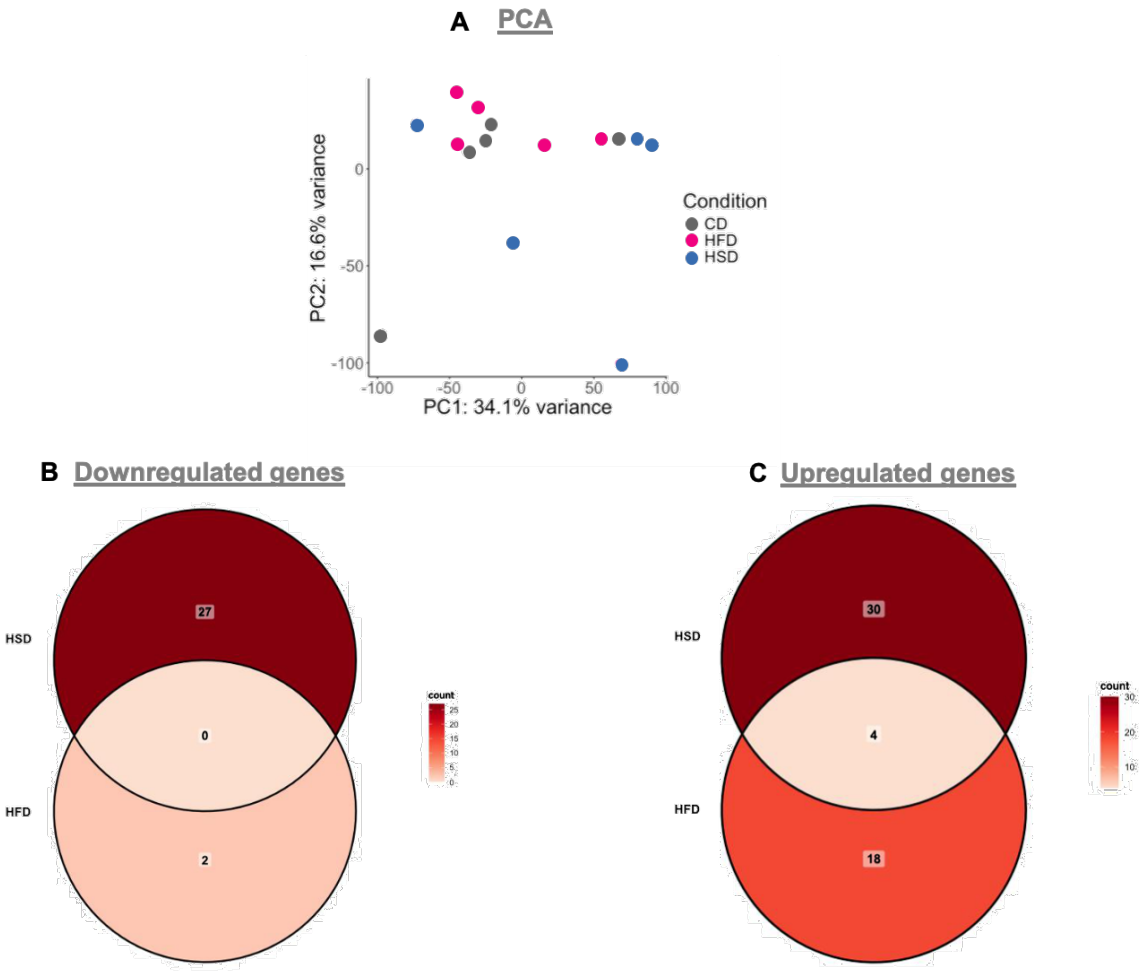



**Figure 12: Role of 7 days HFD and HSD in mother's genetic regulations.** Impact of HFD and HSD in gene profile of mothers. (A) Principal component analysis of mothers. (B) Downregulated genes in HFD and HSD-fed flies relative to CD. (C) upregulated genes in HFD and HSD-fed flies relative to CD. PCA did not revealed a notable distribution. There were 27 genes in HSDm and 2 genes in HFD downregulated. While 30 genes in the HSD group and 18 genes in the HFD group were upregulated (n=5 for all samples).

## 3.2 Impact of maternal obesity on progeny phenotypes

### 3.2.1 The effect of maternal obesity on body weight and circulating glucose levels

The body weight of the flies was measured longitudinally to investigate whether maternal obesity influenced weight gain in the progeny. In male progeny, lower body weights in HFDm and HSDm were seen by day 0. At day 10, both HFDm and HSDm showed a significantly higher body weight. After 50 days, there was no statistical difference (Figure 13). There was no apparent difference in body weight between female progeny on days 0 and 10, while at day 50, HFDm females had a decreased body weight (Figure 13).

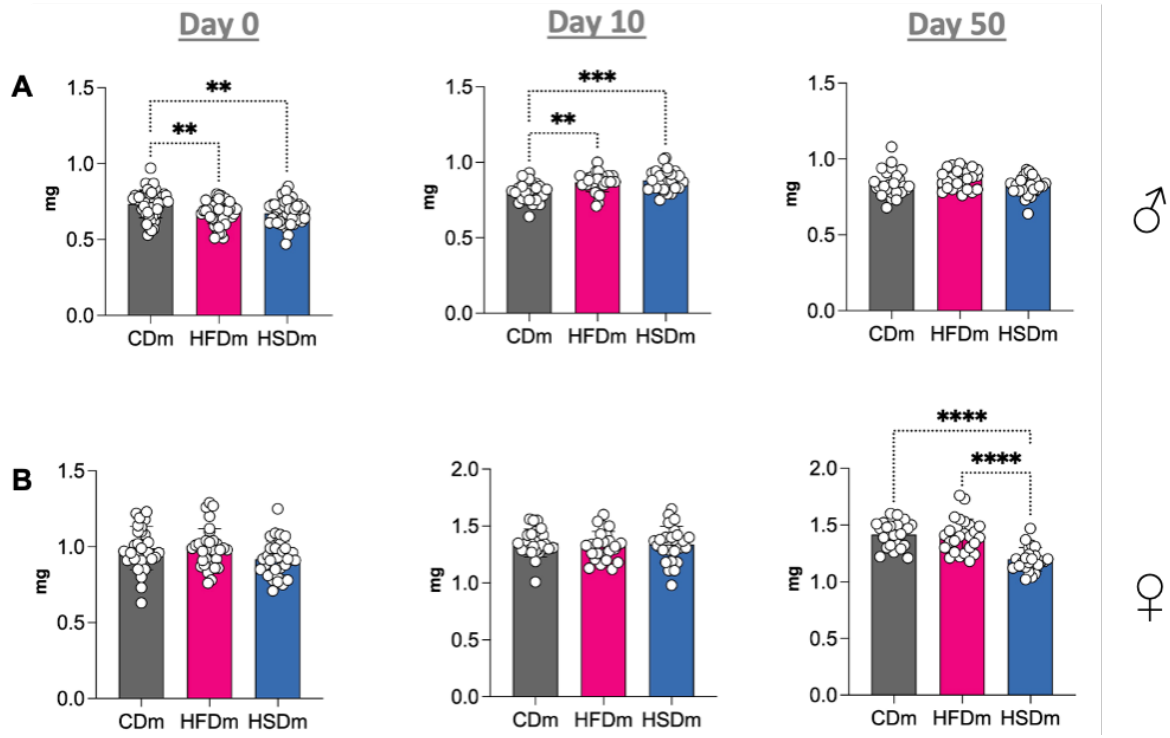



**Figure 13: Longitudinal body weights of progeny.** Bar graphs show progeny body weights for day 0, day 10, and 50, respectively. (A) results of males, (B) results of females. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-fat diet-fed mothers, HSDm: Progeny of high-sugar diet-fed mothers. Although, HFDm and HSDm males had diminished weights on the day 0, drastically raised on the day 10 and were steady on the day 50. HSDm females' body weights were solely declined, while HFDm stayed steady on the day 50. Statistical differences were tested by one-way ANOVA using GraphPad Prism 10. Statistical significance levels were as follows: * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and **** for $p < 0.0001$ (n=45, 25, 25 for days 0, 10 and 50 male progeny respectively, n=32, 24, 22-25 for days 0, 10 and 50 female progeny respectively).

As obesity is often related to increased blood sugar[44], such a condition might also influence progeny circulating glucose levels. A calorimetric assay was conducted to determine whether maternal diet was associated with circulating glucose levels in progeny. There was no statistical difference in the glucose levels of male and female progeny at day 0, day 10, or day 50 (Figure 14).

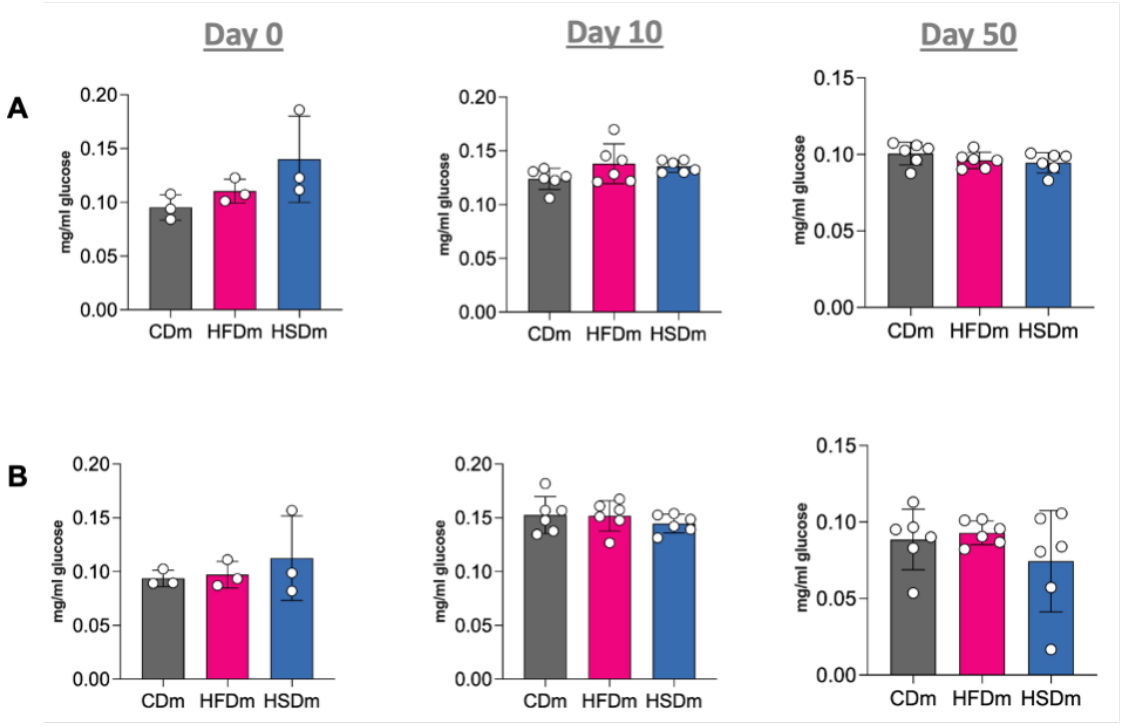



**Figure 14: Longitudinal glucose levels of progeny.** Bar graphs indicate glucose levels of progeny for day 0, day 10 and day 50, respectively. (A) results of males, (B) results of females. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-fat diet-fed mothers, HSDm: Progeny of high-sugar diet-fed mothers. Glucose levels of the progeny were comparable for days 0, 10 and 50 for both males and females. Statistical differences were tested by one-way ANOVA using GraphPad Prism 10. Statistical significance levels were as follows: * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and **** for $p < 0.0001$ (n=3, 6, 6 for days 0, 10 and 50 male progeny respectively, n=3, 6, 6 for days 0, 10 and 50 female progeny respectively).

### 3.2.2 Climbing performance of the progeny

In order to investigate locomotor ability and fitness of progeny, a climbing assay was performed. Female HFDm and HSDm progeny did not represent any detectable difference compared to their control counterparts, thereby indicating no influence caused by maternal obesogenic condition for the female progeny. In contrast, male progeny represented similar climbing performances to controls at day 0 and day 10 of the test, indicating no acute influence of maternal obesity. By day 50, HFDm and HSDm males exhibited a dramatic decrease in climbing ability; considerably, the effects of the maternal diet on locomotor ability declined in an age-dependent manner (Figure 15).

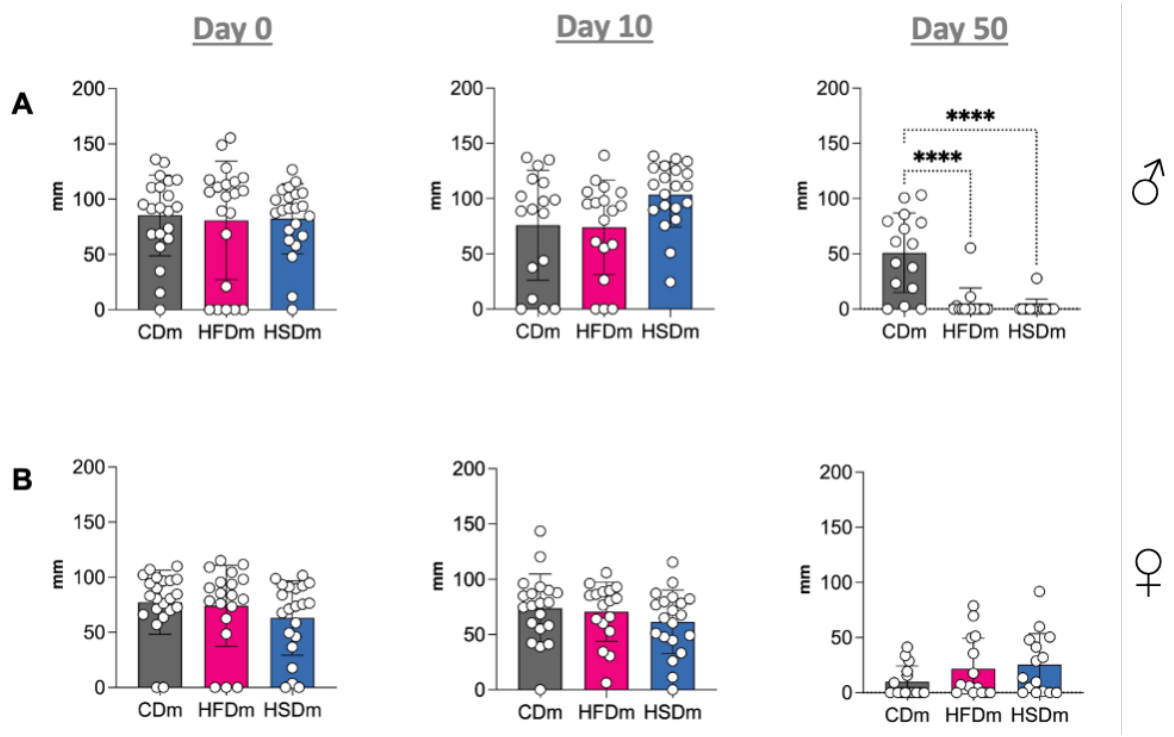



**Figure 15: Longitudinal climbing performance of progeny.** Bar graphs indicate the climbing performance of progeny for days 0, 10, and 50, respectively. (A) male progeny, (B) female progeny. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-fat diet-fed mothers, HSDm: Progeny of high-sugar diet-fed mothers. Climbing ability of female progeny were comparable for the days 0, 10 and 50 for females. Although, male progeny was also comparable on the days 0 and 10, both HFDm and HSDm shown reduced climbing ability on the day 50. Statistical differences were tested by one-way ANOVA using GraphPad Prism 10. Statistical significance levels were as follows: * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and **** for $p < 0.0001$ (n=21-22, 17-20, 15 for days 0, 10 and 50 male progeny respectively, n=19-22, 17-20, 21-22 for days 0, 10 and 50 female progeny respectively).

Both HFDm and HSDm not only represented significant differences in the climbing ability of progeny, which was tested after 6 seconds. Additionally, most male flies failed to climb from the bottom of the tube after 2 min (Figure 16).

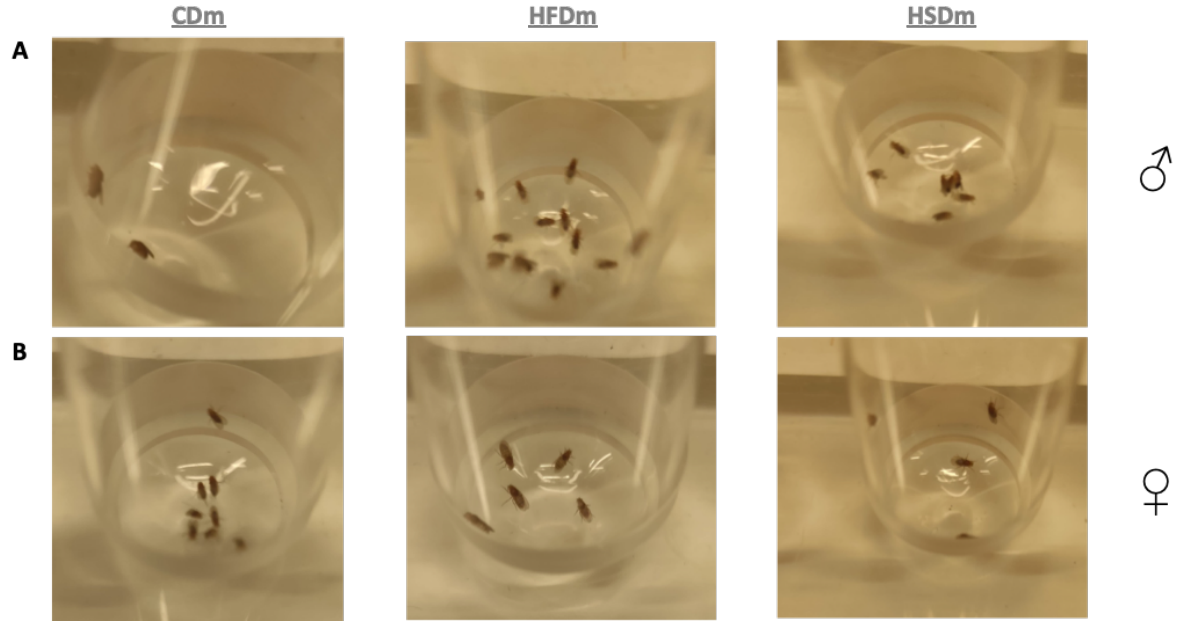



**Figure 16: Aged flies that could not climb for 2 minutes.** Pictures indicate day 50 flies that could not climb for 2 minutes. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-fat diet-fed mothers, HSDm: Progeny of high-sugar diet-fed mothers. (A) pictures of males, (B) pictures of females.

### 3.2.3 Effect of maternal obesity on the progeny's longevity

Physiologically, *Drosophila* flies that are subjected to a high-caloric diet have reduced longevity[84,121] as well as suppressed resistance to various kinds of diseases[84,122]. Diets high in fat[49,73] and sugar[5,123] can influence not only the survival of the individuals but also that of the progeny. The impact of maternal obesity on progeny survival was investigated by measuring longevity in white mutant ($w^{1118}$) flies. Longevity significantly declined solely in HFDm males, while female HFDm stayed steady. There were no differences for HSDm in both female and male progenies compared to CDm (Figure 17).

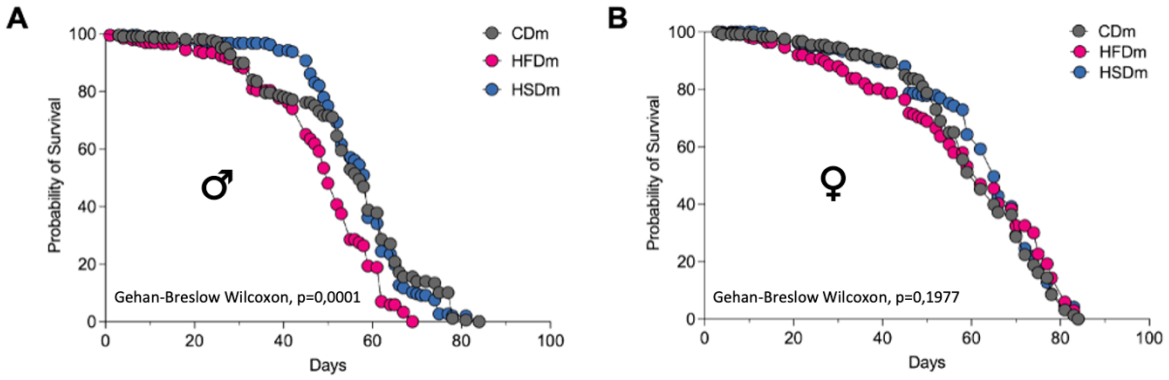



**Figure 17: Longevity of the progeny.** Survival curves indicate the longevity of wild-type progeny. (A) results of males, (B) results of females. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-fat diet-fed mothers, HSDm: Progeny of high-sugar diet-fed mothers. Statistical differences were tested using GraphPad Prism 10. Gehan-Breslow Wilcoxon, p=0,0001, median ages of CDm, HFDm and HSDm were 57, 50, and 59, respectively, in male progeny. Gehan-Breslow Wilcoxon, p=0,1977. For female progeny, the CDm, HFDm, and HSDm medians were 62, 62, and 65, respectively (n=187-195 for male progeny, n=210-225 for female progeny).

### 3.2.4 The role of maternal obesity in axonal degeneration

The RNA-seq data indicated that male HFDm reduced axonogenesis, axon guidance and eye development-related processes compared to CDm and HSDm. Therefore, an axonal degeneration model, generated in AG Tavosanis, utilized and measured the axonal integrity of the R7 region, where axons are located densely in the photoreceptors and project to the medulla in Drosophila.[116]. Similar to RNA-seq findings, while neither HFDm nor HSDm females and HSDm males had comparable termini count relative to CDm, HFDm male flies showed axonal degeneration within the R7 region. (Figure 18). These findings also highlight the importance of sex and diet sources in maternal obesity.

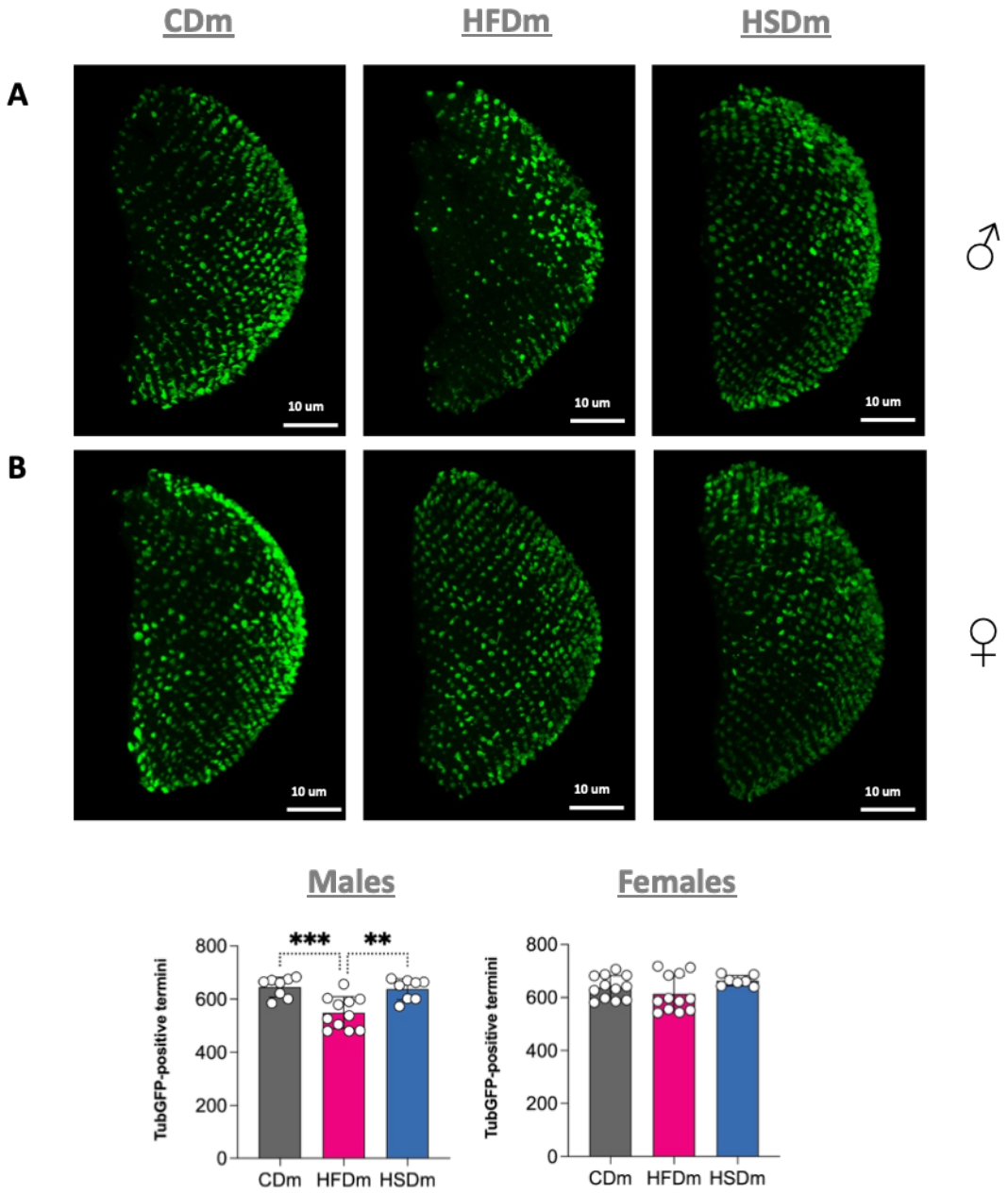



**Figure 18: The light-induced axonal degeneration in progeny.** The figure indicates axonal degeneration in progeny. (A) males, (B) females. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high fat, HSDm, and high-sugar. Although, female progeny had a comparable termini count, males in HFDm had a reduced axonal integrity. Scale bar=10µm. Statistical differences were tested by one-way ANOVA using GraphPad Prism 10. Statistical significance levels were as follows: * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and **** for $p < 0.0001$ (n=8-11 for male progeny, n=7-12 for female progeny).

### 3.2.5 Transcriptional changes in progeny born to HFD- and HSD-fed mothers

To investigate genetic regulations resulting from maternal diet, RNA-seq of the progeny at days 0, 10, and 50 were examined for both sexes separately. PCA was performed to understand the prevalent distinctions in HFDm and HSDm compared to CDm. PCA results showed that sex has the most significant effect among all factors, followed by age as the main driver (Figure 19-A, B, C). Moreover, when age and sex factors were removed, diet-specific clustering was detected in male HFDm and HSDm progeny (Figure 19-D). These clustering effects were less pronounced in female progeny (Figure 19-E).

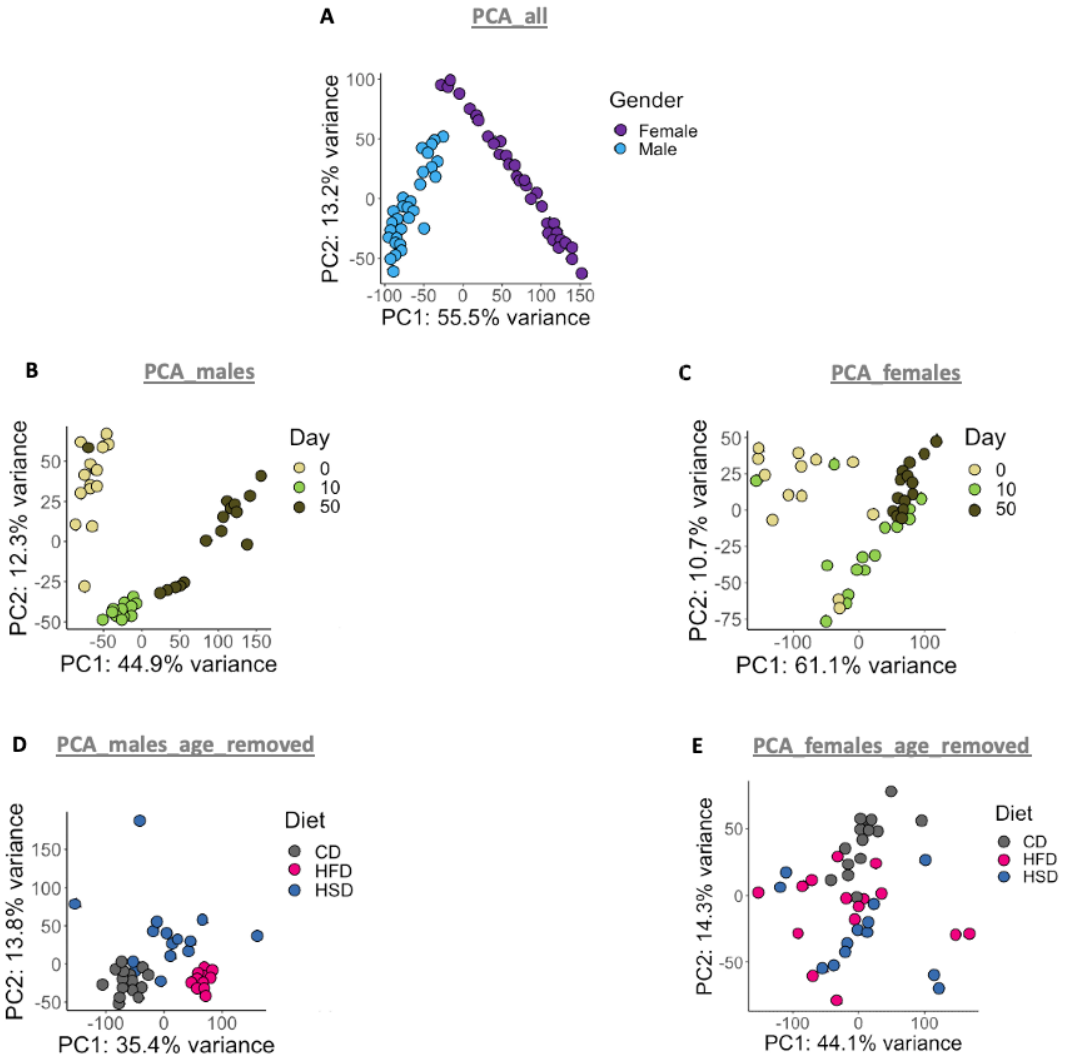



**Figure 19: Principal component analysis of the progeny.** The figure demonstrates the principal component analysis of the progeny. (A) PCA of all samples, (B) PCA of all males, (C) PCA of all females, (D) PCA of removed day factor in males, (E) PCA of removed day factor in females. PCA distributions were only evident in day 50 males of HFDm. Once the age factor was removed, the diet effect was perceptible in male progeny but not females (n=4-5 for all samples).

The present investigation into the PCA and total effects of all parameters, including age, diet and sex, across all samples has uncovered novel genetic changes. When the main day effect was examined, both HSD and HFD progeny increased in direct proportion to age for both sexes, as expected. However, the main diet effect was particularly intriguing and had a more significant effect than HFD in both female and male progeny correlated with age (Figure 20).

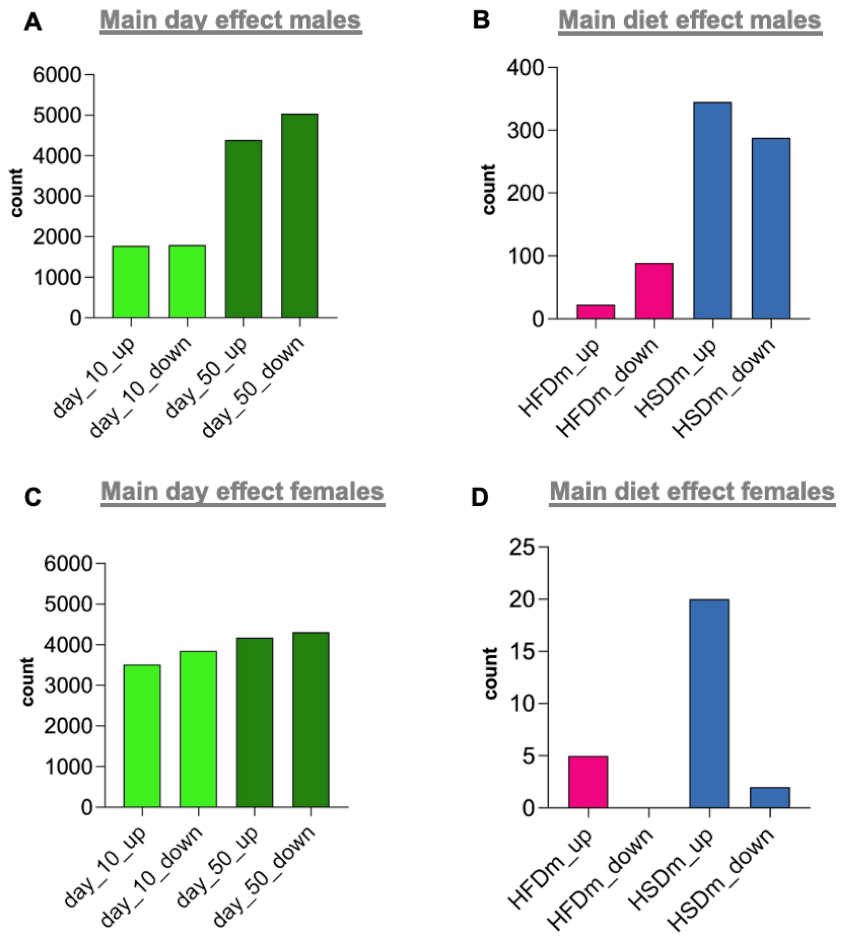



**Figure 20: The main transcriptional effects in HFDm and HSDm progeny.** The figure shows the main effects of progeny on RNA-seq results. While the main diet effect indicates only the impact of the diet variables across all data sets, the main day effect indicates the impact of days 10 and 50 relative to day 0. (A) Main day effects in male progeny, (B) main diet effects in male progeny, (C) main day effects in female progeny, (D) main diet effects in female progeny. The main diet effect was more pronounced in flies on an HSDm and correlated with age.

The total effect parameter, which measured the combined impact of diet and age, revealed that the effect increased in male progeny from the HFD and HSD groups. Interestingly, the effect was more pronounced in female progeny from both dietary groups, peaking on day 10 and showing the most significant effect at this time point (Figure 21).

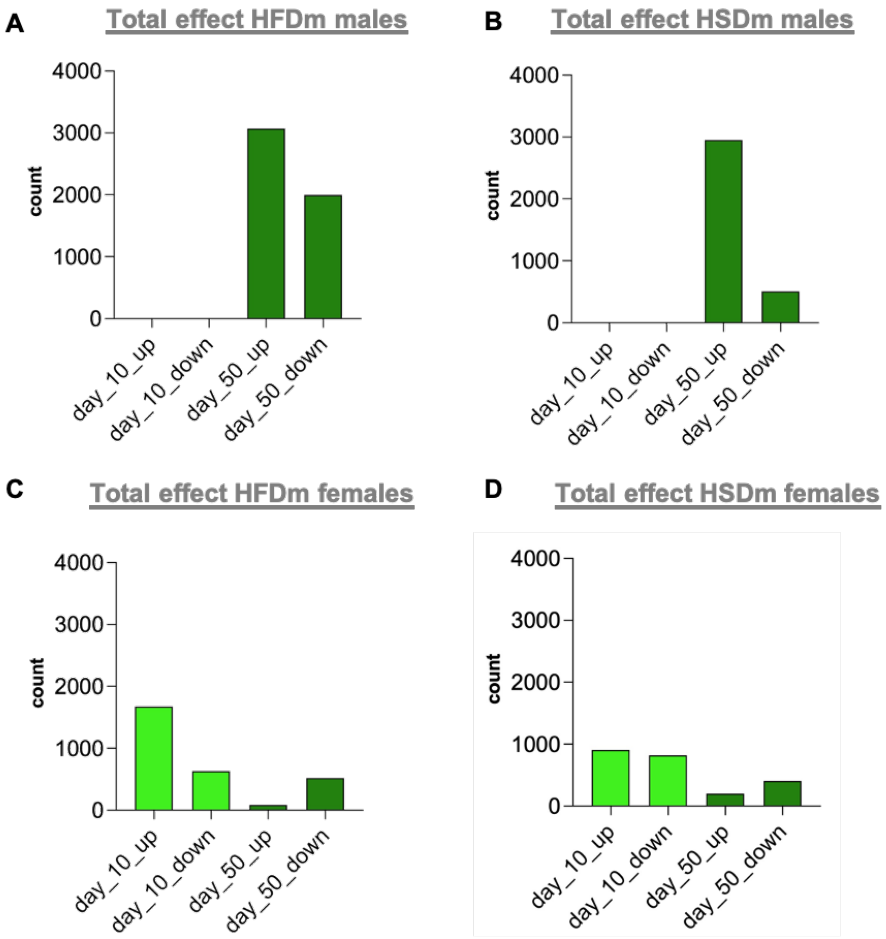



**Figure 21: The total effects of progeny.** The figure shows the total effects of progeny on the RNA-seq results. The total effect indicates the impact of diet and the variables age variables days 10 and 50, relative to day 0. (A) Total day effects in male progeny, (B) total diet effects in male progeny, (C) total day effects in female progeny, (D) total diet effects I in female progeny. The total effect was more significant in day 50 HFDm males and day 10 HSDm females.

Next, the unique and shared genes were investigated across all time points for both genders in a diet-specific manner. No gene was shared longitudinally on days 0, 10, and 50 for up- and downregulated genes in the male progeny. In addition, DEGs in male progeny show the most prominent change on day 50 in up and downregulated genes. In the HFDm group, 15 genes were upregulated on day 0, only 2 genes on day 10 and 2770 genes on day 50 compared to CDm (Figure 22-A), while this situation was detected as 219, 3 and 2153 genes in the HSD group (Figure 22-B), respectively. Similarly, 122 genes were downregulated on day 0, only 6 genes on day 10 and 935 genes on day 50 compared to CDm (Figure 22-C), while this situation was 209, 1 and 220 genes in the HSDm group (Figure 22-D), respectively.

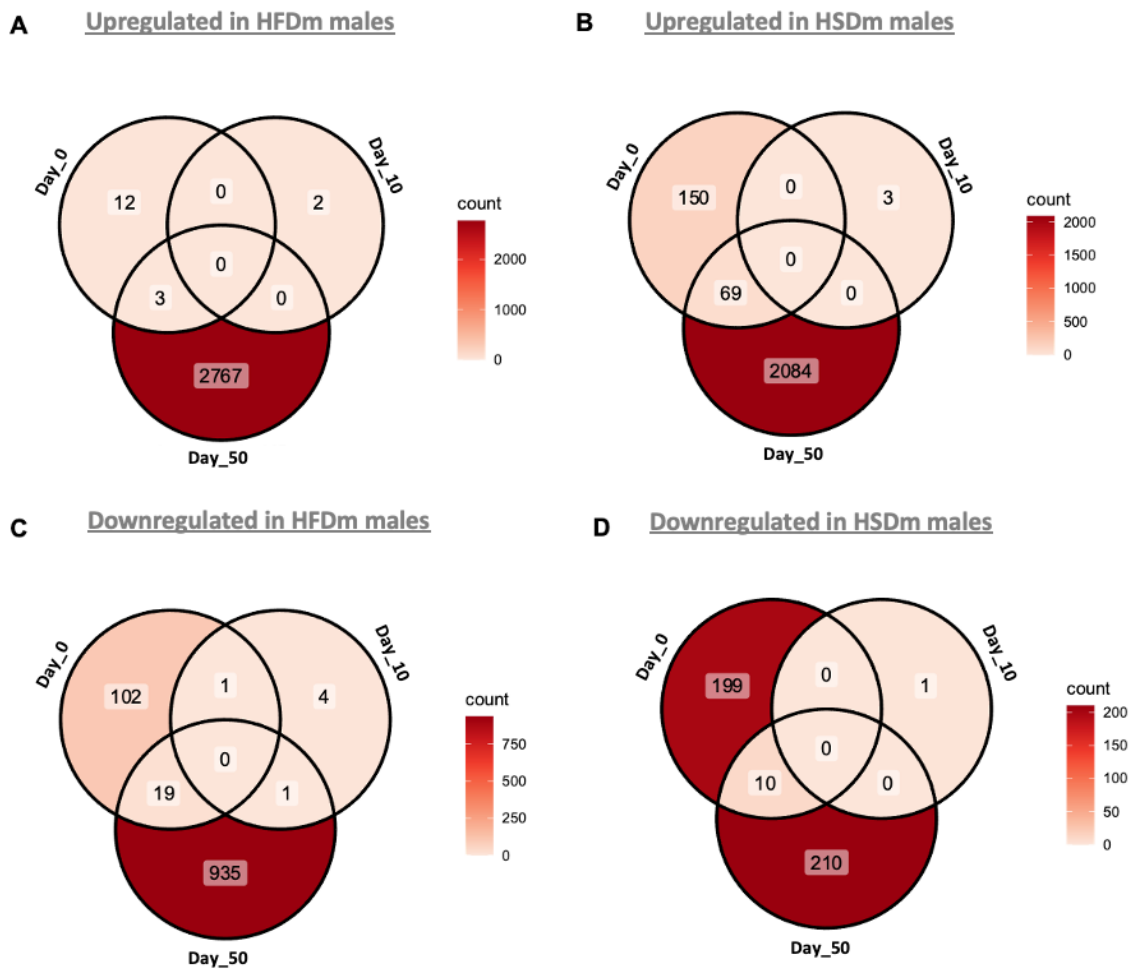



**Figure 22: Venn diagrams of shared genes across all time points in males**. Venn diagrams demonstrate unique and shared genes across all time points in males. (A) Upregulates genes in HFDm, (B) upregulates genes in HSDm, (C) downregulates genes in HFDm, (D) downregulates genes in HSDm.

In female progeny, the total effect became more evident on day 10. The present research revealed that, compared to the CDm group, in the HFDm group, 27 genes were upregulated on day 0, 1303 genes on day 10 and 53 genes on day 50 (Figure 24-A), while there were 16, 657 and 277 genes in the HSDm group (Figure 23-B), respectively. Regarding downregulated genes, in HFDm, 2 genes were differentially expressed on day 0, 1180 genes on day 10 and 449 genes on day 50 (Figure 23-C), while there were 1, 550 and 357 genes in the HSDm group (Figure 23-D), respectively.

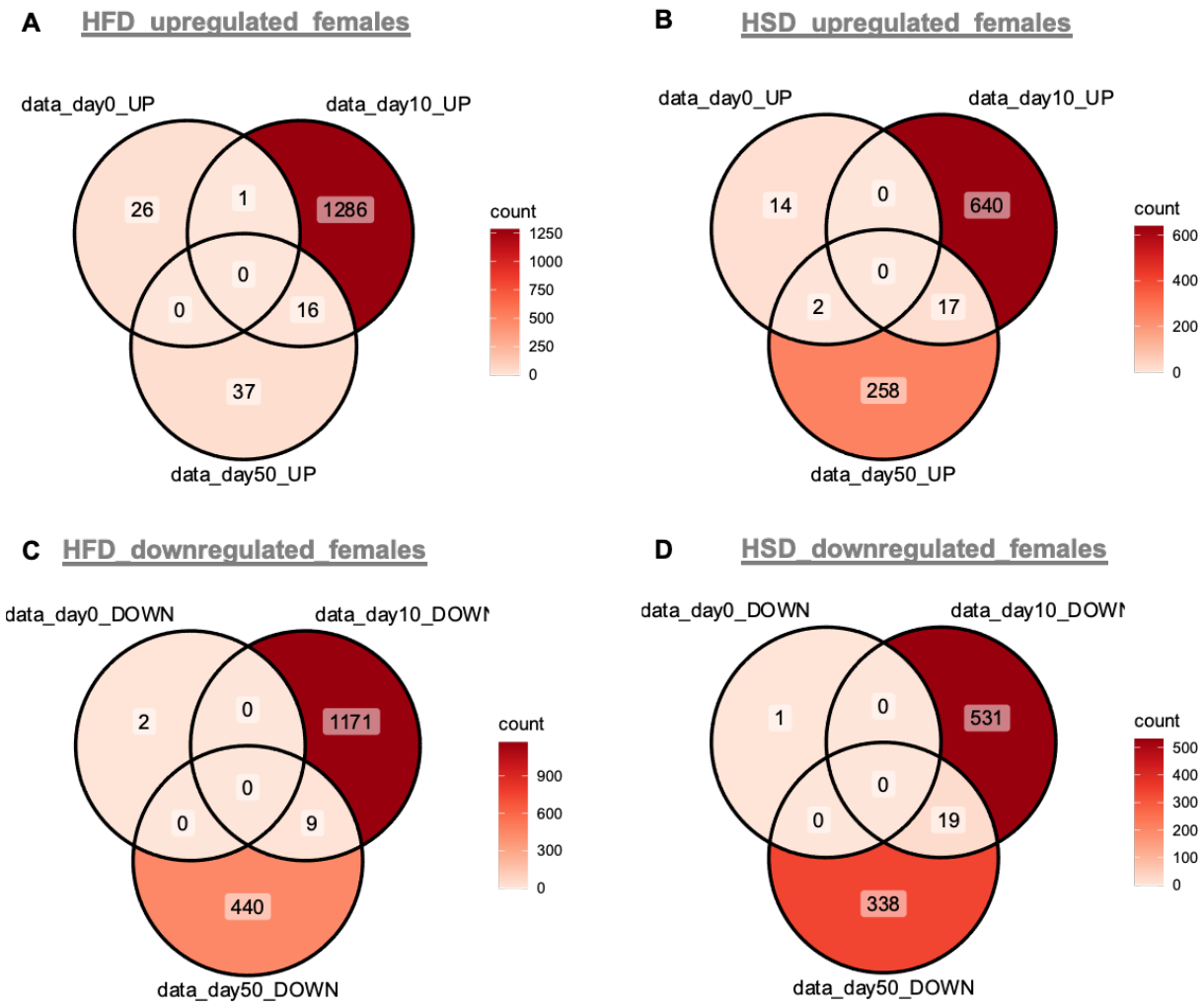



**Figure 23: Venn diagrams of shared genes across all time points in females.** Venn diagrams demonstrate unique and shared genes across all time points in females. (A) Upregulates genes in HFDm, (B) upregulates genes in HSDm, (C) downregulates genes in HFDm, (D) downregulates genes in HSDm,

To understand which molecular mechanisms are affected in the offspring, KEGG and GO enrichment analyses were performed. In HFDm male progeny, KEGG and GO terms were enriched on days 0 and 50 for downregulated genes. The analyses revealed downregulation in the immune system and axonogenesis related terms in male HFDm. Most of the downregulated genes were enriched on day 50 for terms such as hemocyte migration (GO:0035099), positive regulation of defense response to bacterium (GO:1900426), receptor signaling pathway via JAK-STAT (GO:0007259), regulation of innate immune response (GO:0045088), KEGG Toll-like receptor signaling pathway, sevenless signaling pathway (GO:0045500), axon development (GO:0061564), KEGG axon guidance. The eye development (GO:0001654), was enriched on day 0. The upregulated genes in HFDm males were enriched only on day 50 for terms such as cilium assembly (GO:0060271), microtubule bundle formation (GO:0001578), protein targeting to mitochondrion (GO:0006626), spermatid differentiation (GO:0048515), sperm competition (GO:0046692), protein targeting (GO:0006605), pyruvate metabolic process (GO:0006090), ADP metabolic process (GO:0046031), KEGG glycolysis gluconeogenesis, KEGG pentose phosphate pathway (Figure 24).

The downregulated genes in HSDm males were intensively enriched on day 50 for terms such as regulation of innate immune response (GO:0045088), hemopoiesis (GO:0030097), protein phosphorylation (GO:0006468), regulation of glucose metabolic process (GO:0010906), triglyceride metabolic process (GO:0006641), axo-dendritic transport (GO:0008088), KEGG Mtor signaling pathway, KEGG neurotrophin signaling pathway. Also, 2 terms, DNA replication (GO:0006260), KEGG cell cycle, were enriched on day 0. Similarly, the most of upregulated genes in HSDm males were enriched only on day 50 for terms such as protein targeting (GO:0006605), sperm competition (GO:0046692), protein targeting to mitochondrion (GO:0006626), microtubule bundle formation (GO:0001578), protein targeting (GO:0006605), KEGG Parkinson’s Disease, KEGG oxidative phosphorylation, KEGG Alzheimer’s Disease, KEGG glycolysis gluconeogenesis. The term, sperm competition (GO:0046692), was enriched on day 0 (Figure 24).

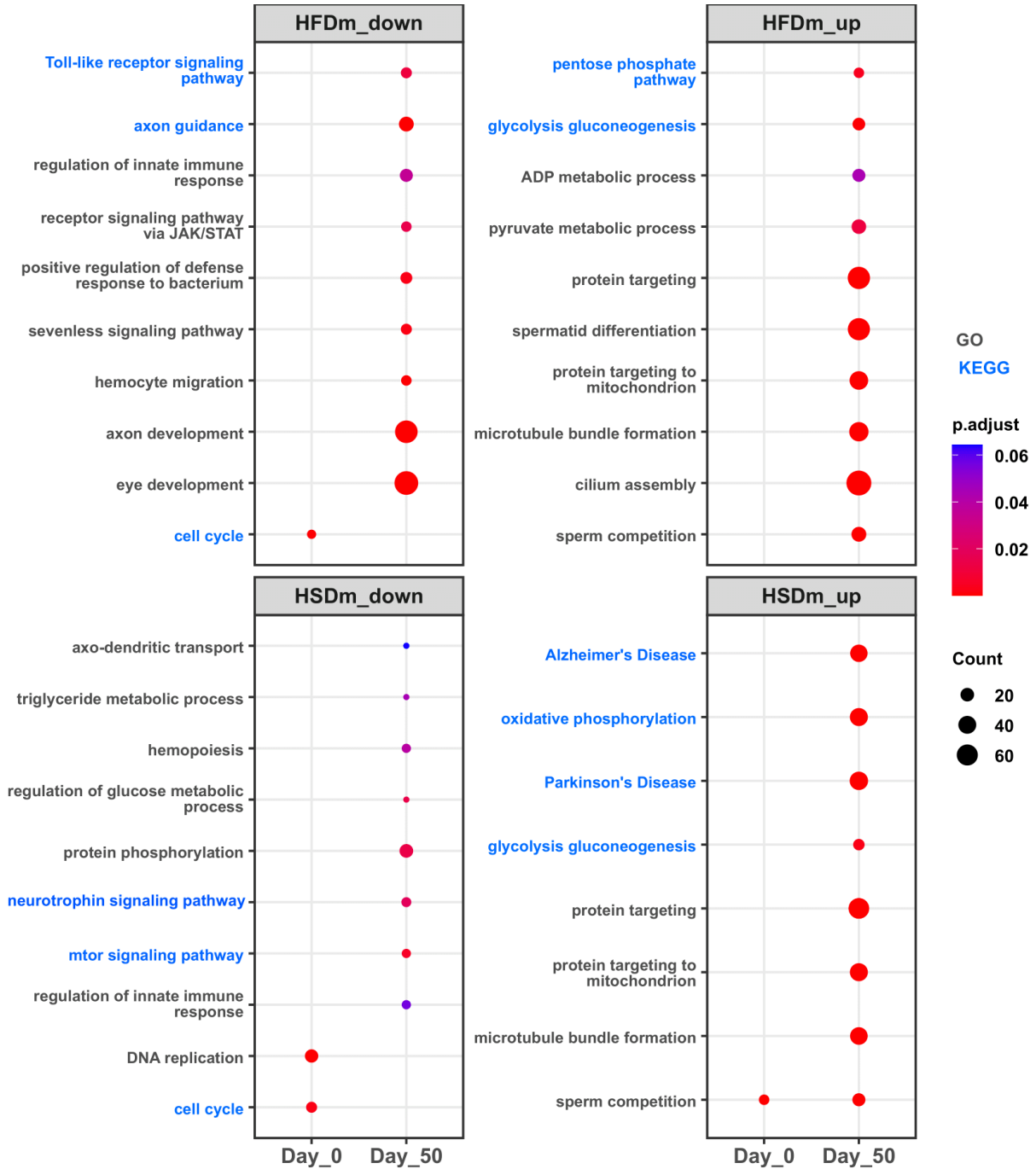



**Figure 24: KEGG and GO terms in the male progeny.** Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) enrichments of differentially expressed genes in the male progeny. The KEGG enrichments were indicated in blue colour. The GO enrichments were shown in grey colour.

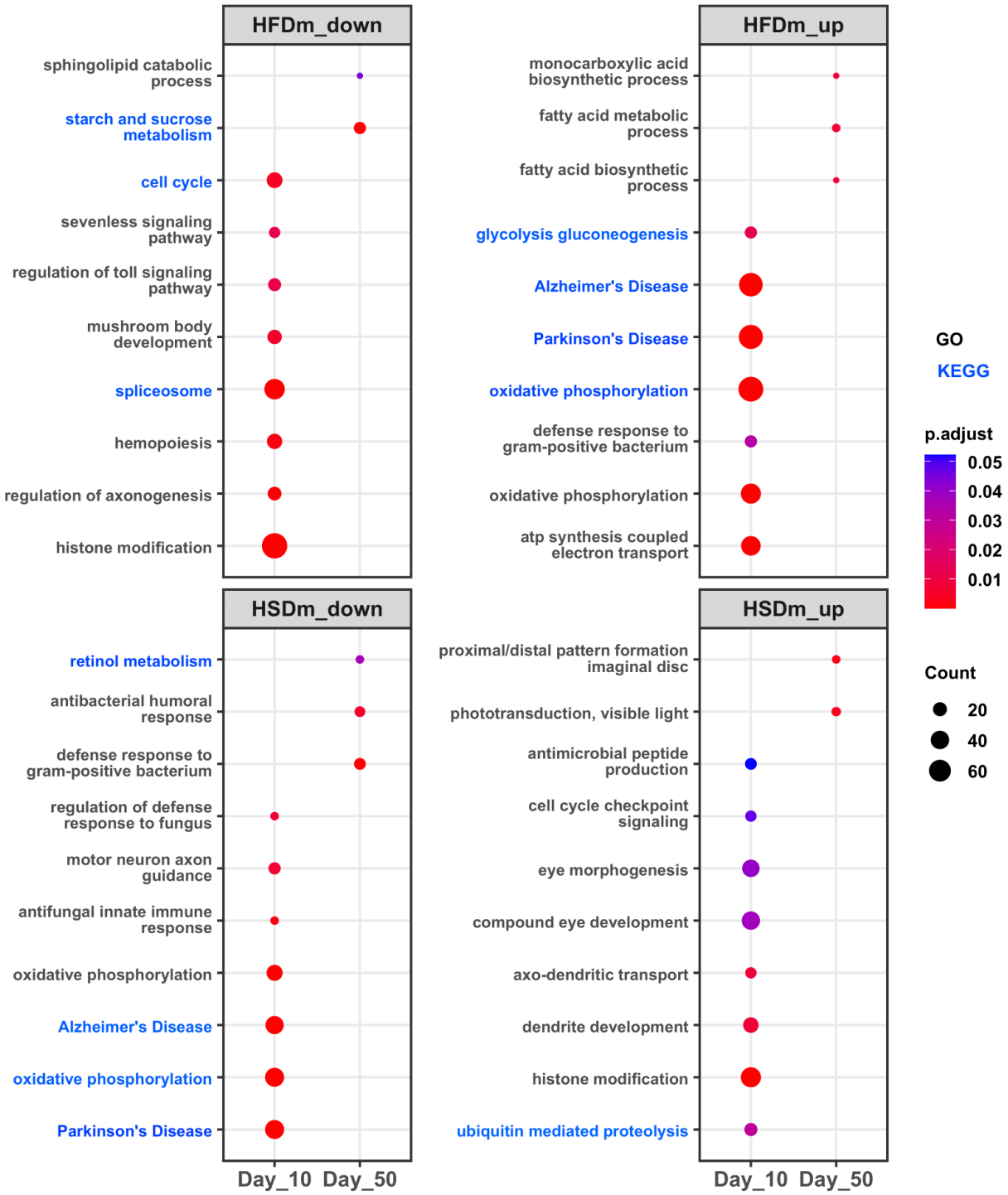
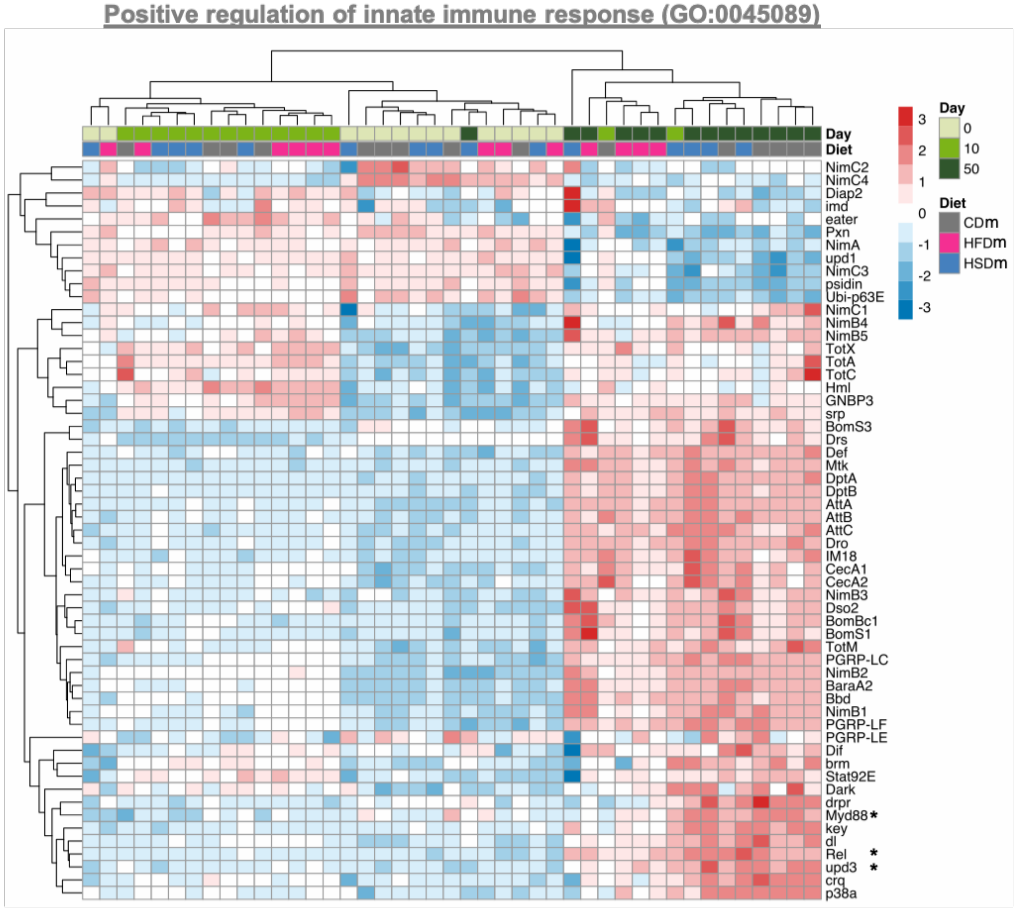



**Figure 25: KEGG and GO terms in the female progeny.** Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) enrichments of differentially expressed genes in the female progeny. The KEGG enrichments were indicated in blue colour. The GO enrichments were shown in grey colour.

KEGG and GO enrichment analyses were also performed in the female progeny, and both HFDm and HSDm female progeny terms were enriched on days 10 and 50. Most of the downregulated genes in HFDm females were enriched on day 10 for terms such as histone modification (GO:0016570), KEGG spliceosome, regulation of Toll signaling pathway (GO:0008592), sevenless signaling pathway (GO:0045500), positive regulation of axonogenesis (GO:0050772), mushroom body development (GO:0016319), hemopoiesis (GO:0030097), KEGG cell cycle. The KEGG starch and sucrose metabolism, sphingolipid catabolic process (GO:0030149), was enriched on day 50. Subsequently, the upregulated genes were analyzed in HFDm female progeny. The upregulated genes in HFDm females were enriched only on day 10 for terms such as ATP synthesis coupled electron transport (GO:0042773), oxidative phosphorylation (GO:0006119), defense response to Gram-positive bacterium (GO:0050830) (Figure 25)

Most of the downregulated genes in HSDm females were enriched on day 10 for terms such as oxidative phosphorylation (GO:0006119), antifungal innate immune response (GO:0061760), motor neuron axon guidance (GO:0008045), regulation of defense response to fungus (GO:1900150), KEGG Parkinson's Disease, KEGG oxidative phosphorylation, KEGG Alzheimer's Disease. The defense response to Gram-positive bacterium (GO:0050830), antibacterial humoral response (GO:0019731), KEGG retinol metabolism, was enriched on day 50. Subsequently, the upregulated genes were analyzed in HSDm female progeny. The upregulated genesin HSDm females were enriched only on day 10 for terms such as histone modification (GO:0016570), dendrite development (GO:0016358), axo-dendritic transport (GO:0008088), compound eye development (GO:0048749), eye morphogenesis (GO:0048592), cell cycle checkpoint signaling (GO:0000075), antimicrobial peptide production (GO:0002775), KEGG ubiquitin mediated proteolysis. The phototransduction, visible light (GO:0007603), proximal/distal pattern formation, imaginal disc (GO:0007449), was enriched on day 50 (Figure 25).

A


Positive regulation of innate immune response (GO:0045089)
Day
Diet
NimC2
NimC4
Diap2
imd
eater
Pxn
NimA
upd1
NimC3
psidin
Ubi-p63E
NimC1
NimB4
NimB5
TotX
TotA
TotC
Hml
GNBP3
srp
BomS3
Drs
Def
Mtk
DptA
DptB
AttA
AttB
AttC
Dro
IM18
CecA1
CecA2
NimB3
Dso2
BomBc1
BomS1
TotM
PGRP-LC
NimB2
BaraA2
Bbd
NimB1
PGRP-LF
PGRP-LE
Dif
brm
Stat92E
Dark
drpr
Myd88 *
key
dl
Rel *
upd3 *
crq
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Day
0
10
50
Diet
CDm
HFDm
HSDm

B

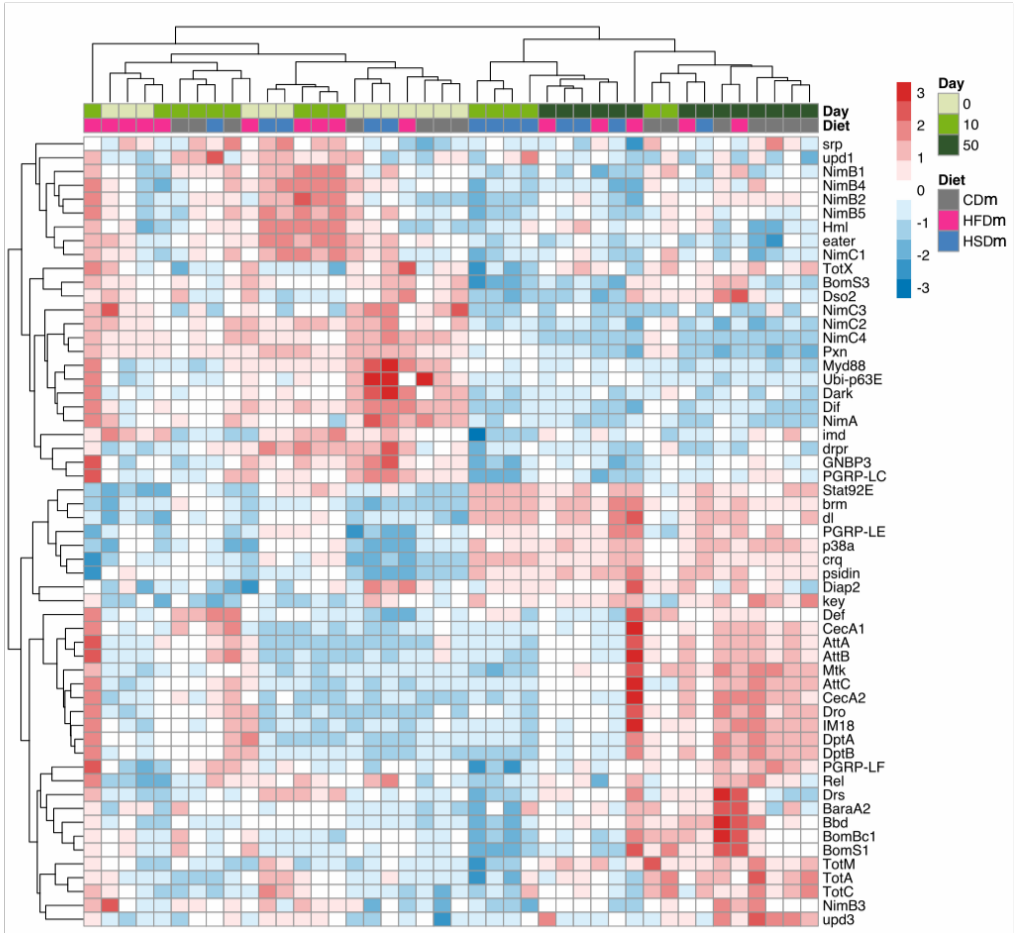
Day
Diet
srp
upd1
NimB1
NimB4
NimB2
NimB5
Hml
eater
NimC1
TotX
BomS3
Dso2
NimC3
NimC2
NimC4
Pxn
Myd88
Ubi-p63E
Dark
Dif
NimA
imd
drpr
GNBP3
PGRP-LC
Stat92E
brm
dl
PGRP-LE
p38a
crq
psidin
Diap2
key
Def
CecA1
AttA
AttB
Mtk
AttC
CecA2
Dro
IM18
DptA
DptB
PGRP-LF
Rel
Drs
BaraA2
Bbd
BomBc1
BomS1
TotM
TotA
TotC
NimB3
upd3
3
2
1
0
-1
-2
-3
Day
0
10
50
Diet
CDm
HFDm
HSDm

**Figure 26: Heatmaps of positive regulation of innate immune response in progeny.** Heat-maps of positive regulation of innate immune response in progeny (A) males, (B) females. The heat map reveled no apparent difference in the gene expression, while the male progeny of HFDm had lower expression for particular genes such as relish (NF-kB) and Upd3.

To understand how the immune system was genetically regulated, the heatmaps of genes related to the positive regulation of innate immune response were generated in female and male progeny (Figure 26). As shown in the heatmaps, while some genes essential in response to pathogens, such as draper (drpr), JAK/STAT (STAT92E), relish, Upd3 and MyD88, declined in the HFDm group compared to CDm and HSDm in day 50 male progeny (Figure 26-A), for female progeny there was not an observable change between groups any time points (Figure 26-A).

In summary, the RNA-seq results revealed the sex and day-specific regulations in the progeny. The major genetic regulations were on day 10 for female progeny and day 50 for male progeny. In addition, PCA revealed a unique clustering of HFDm males. KEGG and GO terms enriched in days 0 and 50 for males and days 0 and 50 for females. Moreover, the KEGG and GO of downregulated genes in the male progeny of HFDm enriched for axonogenesis and immune system-related terms. Furthermore, both upregulated and downregulated genes were examined in both groups to examine how similar and unique gene regulation was shown in HFDm and HSDm groups compared to CDm. As indicated in the figure, while most upregulated genes were shared, 1737 genes were shared, while downregulated genes were highly unique for the HFDm group.

In conclusion, maternal diet affects progeny health in a diet type, age, and sex-dependent manner. Glucose levels and plasmatocyte counts were stable for all groups. Both HFDm and HSDm male progeny had low body weight on day 0, but on day 10 they showed a significant increase compared to CDm, which was equalized on day 50. In addition, body weights were not affected for female progeny on day 0 and day 10, while on day 50, decreased compared to CDm in both HSDm and HFDm. Moreover, the climbing performance did not differ for female progeny on all days 0, 10 and 50 but was significantly reduced in HFDm and HSDm males on day 50. Axonal degeneration and longevity deteriorated only in the HFDm male group. Furthermore, RNA-seq data showed that maternal HFD and HSD had shared and unique transcriptional changes on progeny health longitudinally. When age and diet factors were considered, PCA

results showed that only day 50 HFDm male progeny clustered differently. However, when age and sex factors were removed, the PCA of any female group did not cluster distinctly, while for the males, HFDm clustered distinctly. KEGG and GO analyses also showed that longitudinally significant processes were similarly and distinctly affected among all groups. In female progeny, the majority of terms were enriched on day 10, while in male progeny, they were enriched on day 50. While changes related to axonogenesis and the immune system were downregulated in HFDm male progeny on day 50, changes related to metabolism and spermatogenesis were upregulated in both maternal obesity models. In HSDm male progeny, terms such as innate immune response and hemopoiesis were downregulated, while processes such as Parkinson's Disease, Alzheimer's Disease, oxidative phosphorylation, and glycolysis gluconeogenesis were upregulated. In female progeny, in both HFDm groups, immune system-related processes such as axonogenesis and regulation of Toll signalling pathway were downregulated on day 10, while terms such as glycolysis gluconeogenesis and neurodegeneration-related Parkinson's Disease, Alzheimer's Disease were upregulated.

### 3.4 The role of maternal obesity in the immune response

#### 3.4.1 Role of the maternal diet on plasmatocytes

RNA-seq analyses revealed that MYD88, relish, and Upd3 genes, which play an essential role in immunity and mainly the plasmatocyte functions of male progeny, were downregulated in the HFDm group compared to CDm and HSDm (Figure 26). Moreover, KEGG and GO enriched for immune system related terms especially in the downregulated genes of HFDm males (Figure 24). Thus, plasmatocyte numbers were quantified to understand whether the immune regulations are unique at the molecular level or whether maternal diet also impacts cell density. Thus, to investigate whether immune cell density was affected by maternal obesity, plasmatocyte numbers of HFDm and HSDm flies were quantified. There were no differences between the groups in male flies on days 0, 10, and 50 (Figure 27-A). Similarly, female plasmatocyte counts remained steady on days 0 and 10 (Figure 27-B). The stability in plasmatocyte count for male and female progeny indicated that diet-induced maternal obesity does not influence the density of Drosophila's macrophages.

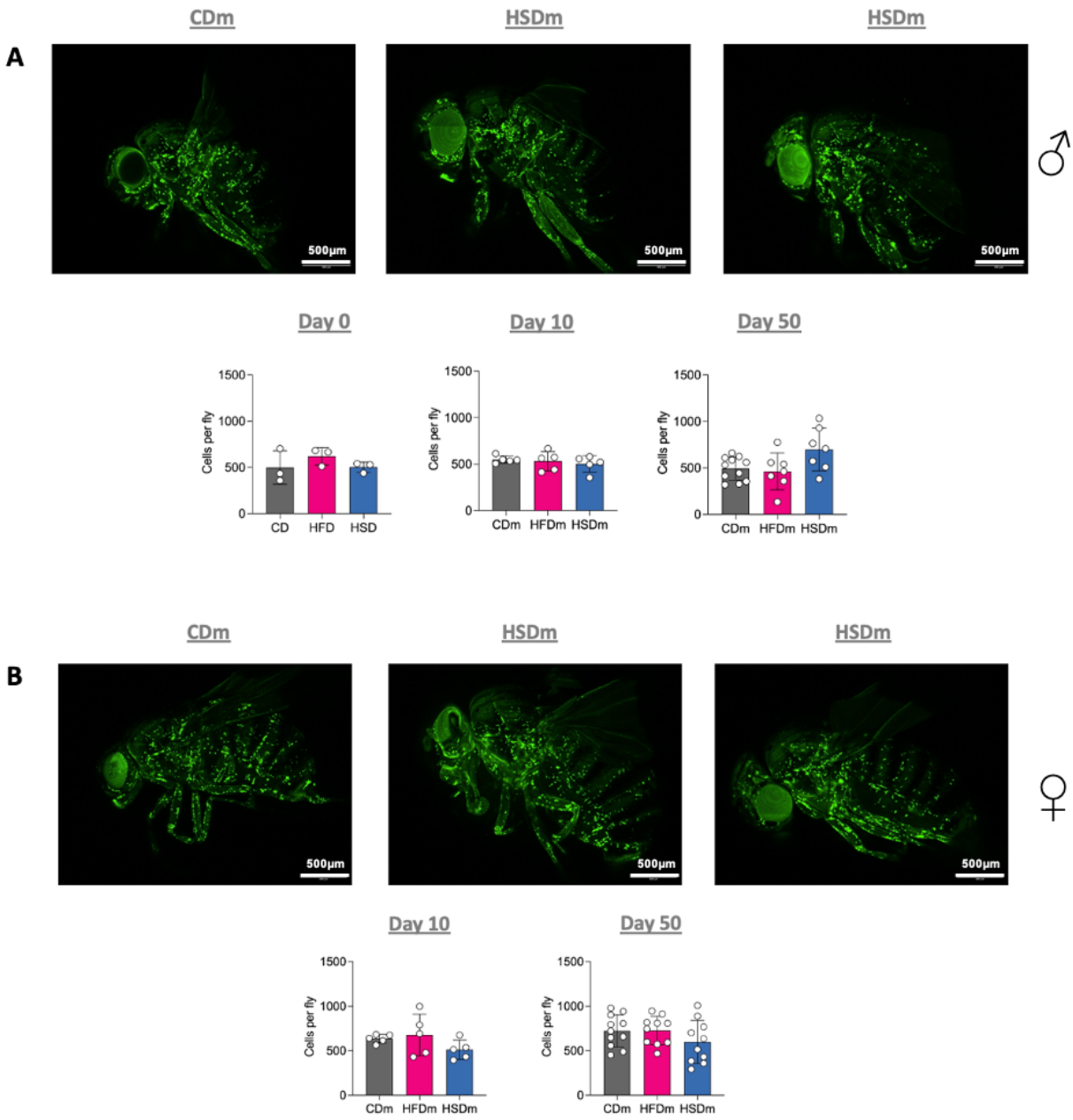


**Figure 27: The plasmatocyte counts of progeny.** The figure illustrates the plasmatocyte counts of progeny. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-fat diet-fed mothers, HSDm: Progeny of high-sugar diet-fed mothers. Scale bar=500µm Statistical differences were tested by one-way ANOVA using GraphPad Prism 10. Both male and female progeny of HSDm and HFDm had a comparable plasmatocytes count for all time points (n=3, 5, 7-11 for days 0, 10 and 50 male progeny respectively, n=5, 10-11 for days 10 and 50 female progeny respectively).

### 3.4.2 Role of the maternal diet on JAK/STAT activation

To reveal the regulation of the fly immune system, particularly the activation of unpaired family groups such as Upd2 and Upd3, JAK/STAT activities progeny was investigated.

The JAK/STAT activity in female progeny was comparable on days 0 and 10. For male progeny, while JAK/STAT activity declined on day 0 for HSDm males, there were no differences on day 10 (Figure 28).

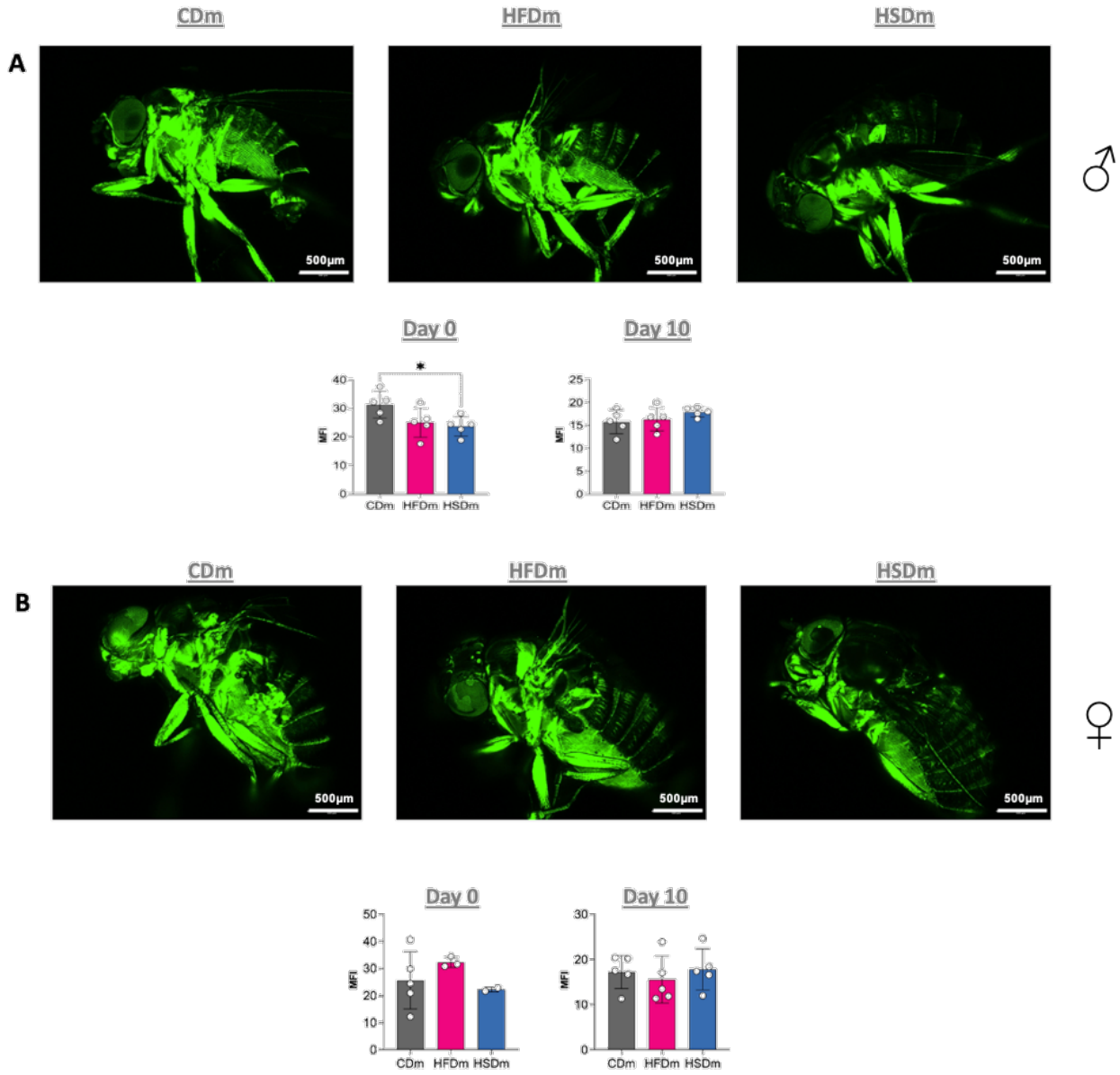


**Figure 28: The JAK/STAT activity of progeny.** The figure shows the JAK/STAT activity of progeny. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-fat diet-fed mothers, HSDm: Progeny of high-sugar diet-fed mothers. Although, female progeny had comparable activity of JAK/STAT for the days 0 and 10, male progeny shown a decline. Scale bar=500µm. Statistical differences were tested by one-way ANOVA using GraphPad Prism 10. The statistical significance level for * is $p < 0.05$ (n=5 for days 0 and 10 male progeny respectively, n=2-5 for days 0 and 10 female progeny respectively).

### 3.4.3 The influence of plasmatocyte-specific knockout of *NF-kB, Upd3* and *Myd88*

In order to investigate the possible impact of maternal HFD and HSD on the progeny's immune machinery, specifically NF-kB, Upd3, and Myd88 were depleted in hemocytes,

and then investigated for survival. The survival assay revealed that, the hemocyte-specific immune deficiency significantly decreased the lifespan of male flies. Specifically, the knockdown of NF-kB drastically decreased the longevity and median age in male flies within the maternal HFD category (Figure 29).

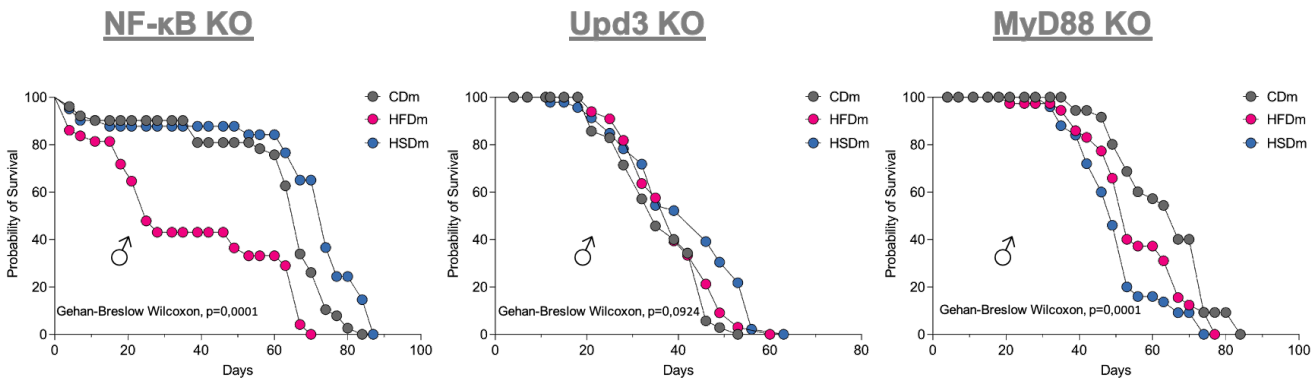


**Figure 29: Longevity of hemocytes specific KO of NF-kB, Upd3 and MyD88.** Survival curves indicate longevity of male progeny of hemocytes specific KO of NF-kB, Upd3 and MyD88, respectively. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-diet-fed mothers, HSDm: high-sugar diet-fed mothers. Statistical differences were tested using GraphPad Prism 10. In the NF-kB KO zhe Gehan-Breslow Wilcoxon, p=0,000, and the median ages of CDm, HFDm and HSDm were 67, 25, 74 respectivelly. In the Upd3 KO the Gehan-Breslow Wilcoxon, p=0,0924, the median ages of CDm, HFDm and HSDm were 35, 39, 46 respectivelly. In the MyD88 KO the Gehan-Breslow Wilcoxon, p=0,0001, and the median ages of CDm, HFDm and HSDm were 67, 53 49 respectivelly.

### 3.4.4 The influence of antibiotics treatment on survival and climbing

Since flies with an impaired immune system have been reported to have reduced longevity and climbing parameters following pathogenic challenge[102,103,124], chronic antibiotic treatment may boost these parameters. In the present investigation, heat maps of immune system-related genes using RNA-seq analysis have demonstrated the downregulation of genes such as Relish, MyD88, and Upd3 in old male flies (day

50) (Figure 26). Moreover, hemocyte-specific NF-kB depletion has been found to worsen longevity (Figure 29). Thus, to unearth how flies' longevity and climbing performance would change in a germ-free environment. The present investigation revealed that a germ-free state boosted longevity and improved the climbing performance of male HFDm (Figure 30).

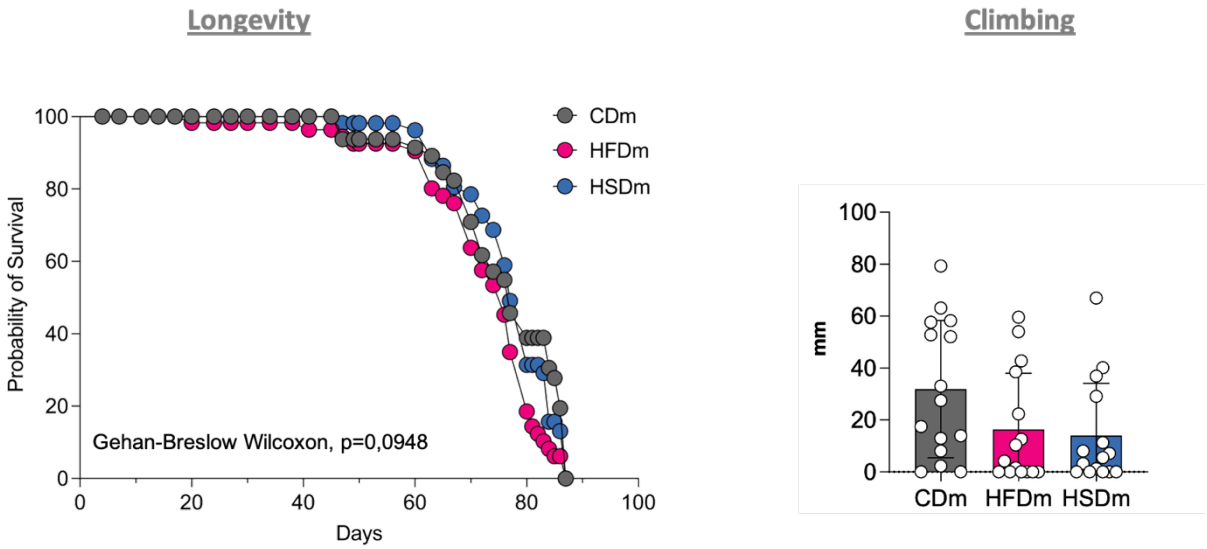


**Figure 30: Influence of the chronic antibiotics in survival and climbing ability.** The survival curve indicates the longevity of wild-type male progeny treated with chronic antibiotics, and bar graphs indicate their climbing performance on day 50. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-fat diet-fed mothers, HSDm: Progeny of high-sugar diet-fed mothers. Statistical differences in longevity and climbing performance was tested by Gehan-Breslow Wilcoxon and one-way ANOVA, respectively, in GraphPad Prism 10. Median ages of CDm, HFDm and HSDm males were 77, 76, 77, respectively. Chronic antibiotics treatment improved the longevity and climbing ability of HFDm (n=52-56 for longevity, n=15 for climbing performance of day 50 males).

### 3.4.5 The role of the bacterial infection on survival and bacterial load

Finally, the post-infection bacterial load and survival tests were conducted to determine the immune system deficiency caused by maternal HFD through exposure to pathogens. The film found that, by exposing pathogens through infecting *E. coli* injections, maternal HFD did not result in an exciting result in bacterial load testing (Figure 31).

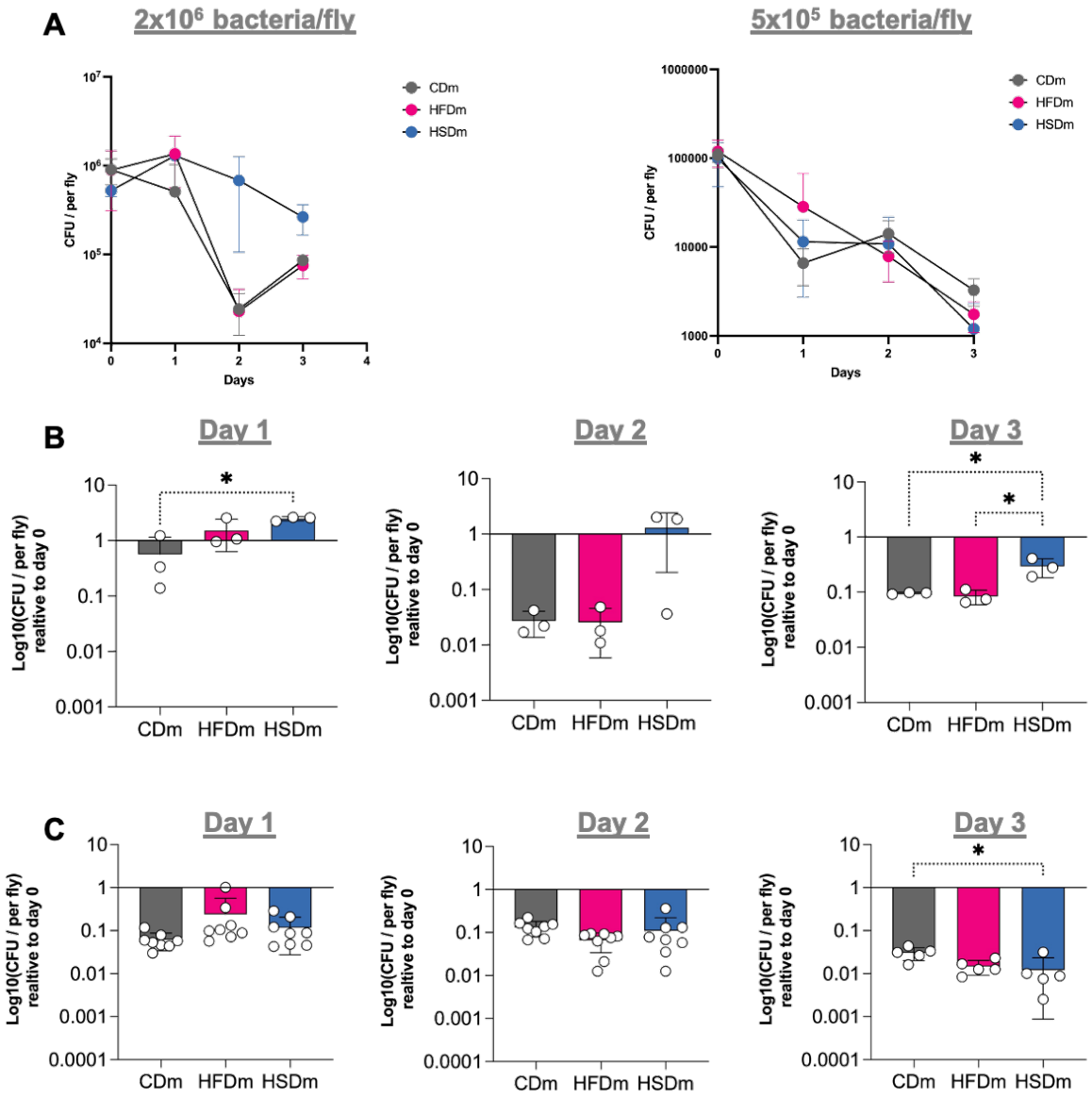


**Figure 31 Post-infection bacterial load.** Bacterial load of male progeny injected with *E. coli.* (A) Alteration of CFU per fly post-infection (B) 2x10$^6$ bacteria/fly, (C) 5x10$^5$ bacteria/fly. CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-fat diet-fed mothers, HSDm: Progeny of high-diet-fed mothers. Statistical differences were tested by one-way ANOVA using GraphPad Prism 10. Statistical significance levels * for is $p < 0.05$ (n=3 for the CFU test following 5x10$^5$ bacteria/fly, n=5-8 for the CFU test following 2x10$^6$ bacteria/fly).

To understand whether flies can survive sepsis, longevity post-injection of bacteria or PBS was investigated. Results demonstrate that the HFDm group represented a more positive survival rate than CDm and HSDm in the survival post-bacterial infection test (Figure 32).

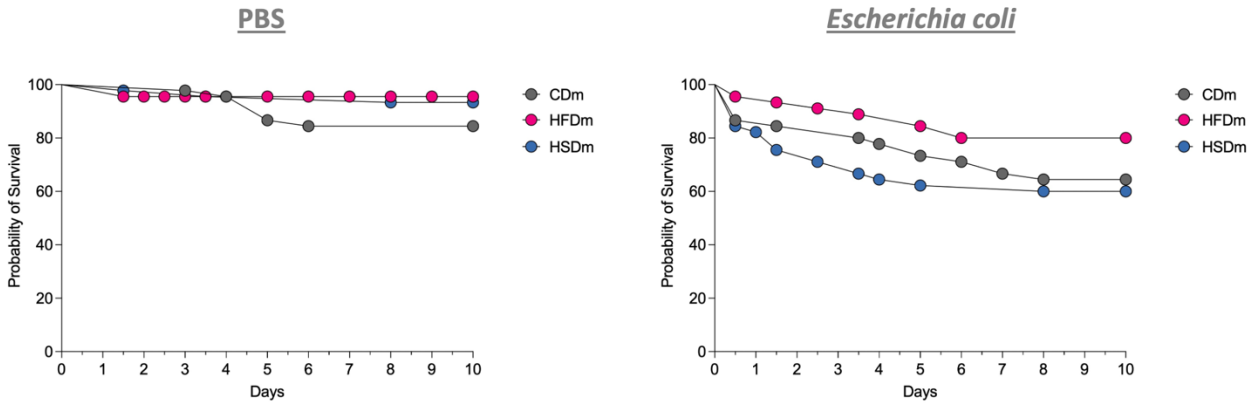


**Figure 32: Post-infection survival.** The figure shows the survival of male progeny post-injection of *E. coli* ($5x10^5$ bacteria/fly). CDm: Progeny of control diet-fed mothers, HFDm: Progeny of high-fat diet-fed mothers, HSDm: Progeny of high-diet-fed mothers (n=45).

Altogether, heat-map of RNA-seq revealed that solely HFDm male progeny blunted gene expression related to positive immune regulation at day 50 compared to CDm and HSDm. GO and KEGG enrichments indicated that, while innate immune response was downregulated in both HFDm and HSDm males, the majority of immune system-related terms such as hemocyte migration, positive regulation of defense response to the bacterium, receptor signalling pathway via JAK-STAT, Toll-like receptor signalling pathway were solely downregulated in HFDm males. Moreover, the longevity of the HFDm group in hemocyte-specific depletion worsened. Furthermore, the chronic antibiotic administration improved the longevity and climbing ability of HFDm males. Thus, on the one hand, these findings imply that the immune system functions were blunted in male HFDm. On the other hand, the plasmocyte counts remained steady and infection of E.coli was not capable to demonstrate immune system dysfunction in HFDm males.

# Chapter 4: Discussion

## 4.1 The roles of HSD and HFD on the metabolic status of *Drosophila*

Hitherto, studies have revealed significant metabolic, immune, and reproductive impacts in both flies and mammals due to HFD and HSD exposure[41,44,84]. HFD exposure leads to lipid accumulation in the fat body of *Drosophila*, similar to the adipose tissue of mammals, resulting in metabolic dysregulations, such as insulin resistance and changes in energy utilization[125,126].

Likewise, consuming HSD increases glucose levels, which in turn promotes the adaptations of insulin-like peptides for maintaining homeostasis, although frequently with metabolic stress[44,57,122]. Studies also indicated that HFD can decline LPC and raise PC, PI, and SM levels, as well as comparable CE levels in mice[127,128]. Thus, lipidomics and glucose levels were examined as vital parameters for the metabolic regulation of CD, HFD, and HSD-fed female flies. While the lipidomics parameters were significantly affected by HFD and HSD compared to the CD (Figures 8, 9), the glucose level remained relatively constant (Figure 11). The present lipidomics results have indicated that HFD and HSD declined only in the PA compared to CD while up-regulating abundant lipid species, including DAG, DAGIS, and LPE (Figure 9). Plus, the 2 shared genes, “FBgn0029172,” and “FBgn0283499” (Figure 12-C), are based on FlyBase reports and are likely impacting the synthesis of di-unsaturated long-chain fatty acids (LCFAs) and regulating the development, respectively[129]. This shared upregulation points toward a common mechanism of dysregulating lipid homeostasis, possibly linked with energy storage and membrane remodelling[81,122,130]. PA is a highly active lipid and influences many homeostatic processes[131]. DAGs are considered vital intermediates of lipid metabolism as they are precursors to TAG synthesis and signaling molecules in different cellular processes, such as proliferation and apoptosis[26]. The fact that diets high in sugar and fat raised a lipid associated with membrane fluidity and curvature, LPE, illustrates the effects these diets may have on macromolecular behaviours at the cell membrane level.

The influence on the lipidome of each diet also showed distinct differences. For example, TAGs were significantly increased in the HSD group which indicated a higher capacity to store energy in fat with increased sugar intake (Figure 9). Next, the HFD group demonstrated raised levels of LPC and its hydroxylated form LPCH (Figure 9).

In addition, the mechanism is poorly comprehended, studies have shown that LPC is linked to obesity[132], and plays a role in lipid inflammatory pathways[125,130,133].

The HFD-fed female flies had a higher amount of CER, HEX-CER (Figure 9), and sphingolipids linked with insulin resistance and metabolic diseases[134]. This unique upregulation of these lipids can support the model of HFD-induced metabolic disorders, which may model some aspects of human metabolic syndrome and, in the case of females, maternal obesity. Next, there was a significant increase in the levels of CAR and PE in the HFD group (Figure 9). Insufficiency of PE can influence mitochondrial function and oxidative phosphorylation[135]. Carnitines have long been reported to have a substantial role in fatty acid oxidation, reflecting adaptive change towards dietary fat challenges by intensifying mitochondrial processing for fatty acid transport[26]. On the other hand, PG-M levels were significantly decreased in the HFD group compared to CD and HSD (Figure 9), indicating a diet-specific lipidome regarding HFD intake.

The HSD group had raised levels of TAG, a vital participant in the transport and storage of cholesterol. (Figure 9). This may suggest a change in the lipidome and metabolism toward maintaining cholesterol homeostasis under HSD circumstances and represent the mechanism of dietary sugars in regulating lipid homeostasis[136].

The levels of specific lipid classes, including LPA, MAG, PC-O, PE-M, PHSE, PIPOS, and SM, revealed no change between dietary groups (Figure 10). These results either indicate that these lipid classes are modulated by particularly robust pathways that are insensitive to perturbation by the diet or are tightly regulated to serve essential cellular functions under variable dietary composition[57,125,130,137].

Besides metabolic effects, dietary intakes also modulate plasmatocytes[96], principal immune cells in *Drosophila*, by induction of states of inflammation similar to low-grade inflammation in the immune responses and functions of cells[138–140]. In contrast, the present findings showed no apparent change in plasmatocyte numbers under both dietary conditions, suggesting that HFD and HSD may not straightforwardly have a similar effect to CD on immune cell population dynamics in the flies (Figure 11).

Reproductive outcomes in *Drosophila* are dramatically impacted by diet: having high caloric value reduces fecundity[43,84] based on endocrine interruptions consecutively to oogenesis[43,51,141]. The present investigation revealed declined productivity in flies exposed to HFD and HSD, with HFD exerting a more significant influence (Figure 11).

Moreover, a previous investigation revealed that HSD consumption may result in systemic insulin opposition and display an ovarian phenotype distinguished by raised lipids and cholesterol in the ovary. Furthermore, HSD declined the ovary dimensions and incapacitated egg development in female *Drosophila*[24].

In summary, HFD and HSD resulted in shared and unique metabolic and reproductive outcomes in females. Dietary HFD and HSD raised lipidome and declined body weight, while glucose levels and the density of plasmatocytes were comparable to CD in *Drosophila*. In addition, productivity solely declined in HFD females. Thus, the source of diet might be an important factor that need to be consider in the obesity.

### 4.2 The effect of maternal diet on the metabolism and phenotype of the progeny

Multiple research studies have demonstrated a vital link between maternal obesity and the developing state of an individual's metabolic disorders in progeny[3,53,73]. Also, maternal nutrition may predispose progeny to metabolic dysfunction, comprising glucose dysregulation and obesity[40,57].

For instance, a single larval diet competent to influence *Drosophila* progeny raised on a high-protein diet experienced metamorphosis quicker, had more elevated reproductive outcomes, and had distinct metabolisms compared to the progeny of adult flies raised lower protein.[72]. Development periods are delayed for progeny raised paternally or maternally on inadequate nutrition compared to those raised on sufficient nutrition [142]. On the other hand, progeny from mothers consuming a high-caloric diet may be programmed to become overweight, possibly because the maternal diet supports this maternal environment and, thus, fetal overnutrition[27,143].

A genotype-specific relation was revealed in egg and pupal dimension induced by HFD, whereas impacts of egg size on adult weight or descendants' egg dimensions were not consistent[4]. Thus, maternal overnutrition may lead to the reprogramming of progeny development toward adiposity and weight gain in *Drosophila*.

In the current investigation, the males are seen to be more prone to undergo modifications in body weight. Initially, males of HFDm and HSDm mothers were lighter at day 0, however, they compensated overall, showing significantly higher weights on day 10 than CDm males, indicating an early-life metabolic adaptation that changes nutrient storage or metabolic programming mediated by maternal diet (Figure 13-A).

Moreover, the weight differences at day 50 normalised across male groups, implying metabolic stabilisation on a standard diet as flies matured. On the other hand, female progeny did not show differences in their weight at days 0 and 10; however, they still showed a significant decline by day 50 when their mothers were on a HFD or HSD (Figure 13-B). Moreover, the majority of genes changed on the day 50 for males (Figure 22) and on day 10 for female progeny (Figure 23), indicating an age and sex-specific impact. Likewise, a previous study also demonstrated that the maternal HFD can shape metabolic parameters in descendants in a sex-specific manner in mice[81]. Therefore, in the present project, the delayed response in the female progeny to the maternal diet may indicate a likely sex-specific metabolic adaptation that affects long-term weight dynamics differently from that in males.

Obesity can also influence the glucose metabolism of the descendants of *Drosophila*[144]. Maternal high-caloric diets during conception were also found to impact glucose metabolism in the mammalian, altering insulin sensitivity and glucose tolerance[47,57,145]. Nevertheless, in the calorimetric assays, the glucose levels for male or female progeny unrelated to the maternal diet were comparable on time the days 0, day 10, and day 50 (Figure 14). These results could be due to a compensatory mechanism in Drosophila to maintain glucose homeostasis, or time of HFD and HSD, 7 days, were not enough to induce glucose metabolism in *Drosophila.*

In *Drosophila*, a commonly used model organism in genetic and developmental studies, the experimental models of the maternal HSD and HFD have provided specific insight into the effects of poor maternal nutrition in descendants. Moreover, Rincel et al. (2016) demonstrated that gestational HFD can also drastically attenuate developmental defects[49].

Furthermore, studies by Musselman et al. (2011)[44] and Heinrichsen et al. (2014)[41] demonstrated the development of obesity and insulin resistance in the progeny of flies fed a high-sugar diet, which changes the transcriptional responses in the metabolic pathways. Indeed, Brookheart et al. (2016)[24] and Birse et al. (2010)[122] have found, respectively, significant changes in gene expression linked to lipid metabolism and stress responses in progeny from *Drosophila* females subjected to a high-fat diet, highlighting thus a persistent imprint on the health of the progeny due to maternal diet.

In conclusion, the present thesis revealed the intricateness of this dynamic system of maternal diet influencing progeny health by showing that early nutrition environments

induce major but subtle metabolic adaptations. Finally, the overall apparent sex and age effect on weight trajectories across the experiment suggest that male and female *Drosophila* adopt different metabolic strategies in response to maternal diet cues. While males showed only transient differences in body weight, females exhibited a retarded weight gain, indicating that both short and long-term effects of maternal diet needed to be considered in female metabolic health. These differences between male and female progeny could be due to sexual dimorphism in adipose tissue functions. For example, a former study indicated that maternal diet could influence the pathology and transcriptomics of adipose tissue in a sex-specific manner[81]. The second set of present findings, suggesting that glucose levels are comparable in the progeny of *Drosophila*, contrasts even further with the common observation of severe glucose dysregulation in mammalian models of maternal obesity. That would suggest, in turn, that there are disctinct metabolic mechanisms operating in *Drosophila* and importance of the species-specific response to the maternal dietary exposures. Furthermore, these findings postulated insight into the relation of metabolic and developmental programming through the influence of maternal HFD and HSD on progeny development and metabolism.

### 4.3 Sexual dimorphism in axonal degeneration and climbing performance of the progeny

Experimental studies, especially with HFD, have revealed that high-calorie diets can affect individuals' brain axonal, neuronal and glial health. For example, HFD can individually diminish the capability of sensorial neurons to function at physiological frequencies and drop mitochondrial membrane potential (MMP) in the mitochondria of axons in mice[146].

Metabolic changes brought on by an HFD may individually affect retinal homeostasis, worsen retinal degeneration, and hasten the pathologic processes of retinal illness by promoting glucose intolerance, gut microbiome dysbiosis, retinal oxidative stress, and inflammation[147]. In mouse models of visual impairment, HFD therapy can replicate the pathologies of some of the primary causes of blindness, including diabetic retinopathy (DR) and age-related macular degeneration (AMD)[148]. HFD consumption in rodents results in obesity but revealed regular retinal function corresponded to chow-fed instances but reduced β3tubulin staining of retinal cross-sections demonstrated more

infrequent deterioration of neuronal functions in the inner plexiform zone[149]. Next, in Meriones shawi, a type II diabetes-like syndrome that results in gliosis and the death of retinal neurons and photoreceptors can be driven through HFD[150]. In addition, in *Drosophila*, a long-term high-fat diet reduces the basal and injury-induced expression of the glia-associated phagocytic receptor Draper, which causes glial insulin resistance and hinders the removal of neural debris[140].

Hence, it is important to understand whether maternal obesity intergenerationally affects brain health, as indicated by the axonal degeneration parameter, which is indicative of an early onset of the neurodegenerative process in the progeny of *Drosophila*. In the present investigation there was no significant difference in axonal degeneration in female HFDm and HSDm, suggesting resilience to maternal diet-induced insults (Figure 18-B). In contrast, male HFDm progeny showed more severe axonal degeneration than those from CDm and HSDm conditions, particularly in the R7 photoreceptor area (Figure 18-A). In addition, GO enrichment in day 50 HFDm male offspring revealed downregulation of genes associated with sevenless signaling pathway, which is important in axonogenesis of R7 region, eye development, axonogenesis, and axonal function (Figure 24). The increased sensitivity raises the risk of axonal injury in male progeny exposed to high-fat diets, indicating a sex-specific susceptibility to maternal HFD. This sensitivity may be mediated by variations in the metabolism of lipids, hormonal impacts or intrinsic properties of males under metabolic stress. Although there are studies examining the effects of maternal diet on progeny brain health, they are limited. Some of the studies have revealed the following findings. Maternal HFD may involve the neurodevelopment of progeny due to chronic inflammation of the maternal gut, adipose tissue, and placenta, reflected by boosted release of pro-inflammatory cytokines in the circulation of the progeny[55]. Maternal obesity could influence cognitive and behavioural features in the progeny, and maternal nutrition itself may influence the development of such an inflammatory milieu[3]. Nutritional factors, such as HFD during the perinatal period, induce the development of cerebrovascular and microglial anomalies in mice's offspring, further strengthening this effect. Such disorders may result in exaggerated neuroinflammatory responses with concomitant behavioural deficits[67].

As reported by Bordeleau et al. (2021)[59,63], maternal nutrition has a considerable impact on neurological outcomes, myelin organization, and social memory, with potentially broad implications for neurodevelopmental problems. In addition to this, the

HFD-exposed mothers' progeny had depressive-like behaviours together with a reorganization of the myelin myeloarchitecture in the prefrontal cortex[80].

These findings have implications for investigating the neural consequences of maternal obesity and indicate enhanced vulnerability for neurodegenerative conditions in the later life of the progeny through axonal degeneration in *Drosophila*. The vulnerability to axonal degeneration was sex-specific and fits into general observations within neurobiology that males and females are different in their susceptibility to neurological disease.

In the present investigation, the HFDm and HSDm groups revealed sexual dimorphism in climbing performance, and by day 50, both male groups had significantly declined locomotor ability (Figures 15, 16). The decline in climbing performance of male progeny indicates a progressive loss in locomotion due to maternal diet in male progeny, which revealed that long-term maternal diet-induced obesity may influence locomotor health in a sex-specific manner. RNA-seq analysis also revealed that the main dietary effect was more pronounced in HSDm flies and correlated with age (Figure 20), but the total effect was more significant in day 50 HFDm males and day 10 HSDm females (Figure 21). Thus, the long-term and permanent effect of maternal diet may predominantly affect the male progeny of HFDm. A study indicated that, the chronic exposure to an HFD can result in a rise in body weight and diminished climbing ability in *Drosophila*[126]. HFD leads to worsened climbing capability in aged *Drosophila*[84]. Both maternal HFD and HSD can impact the locomotion in the offspring[40]. Next, Franskowkska et al. (2023) demonstrated that the maternal HFD caused long-lasting adaptations, as evidenced by the decrease in both immature and mature oligodendrocytes in the offspring's prefrontal cortex and lengthened the period of locomotor activity in the forced swimming test[80]. Moreover, a systematic review revealed that maternal HFD can both boost and diminish the locomotion in the descendants[152].

In conclusion, there is existing evidence linking sexual dimorphism to differences in disease susceptibility, brain pathology and cognitive outcomes[4,27,40]. The sex-specific cognitive deficits and neuronal pathology occur in males rather than females in *Drosophila* models of Alzheimer's disease[151]. Likewise, in the current study showed that maternal obesity has a sex-specific impact on the climbing ability and axonal degeneration of offspring in *Drosophila*. Additional research should examine the

identified disease in preclinical animal experiments in order to shed light on this process.

### 4.4 Influence of the maternal diet on the longevity of progeny

Obesity is closely linked with deficient fitness and function and diminished human longevity[130]. Dietary ingredients particularly influence longevity in flies[153]. Studies have demonstrated the adverse relationship between high caloric diets and longevity[84], which is undeniably principally mediated by metabolic health and a greater probability of developing persistent medical conditions in the long term[3,39,73]. Obesity caused by HSD or HFD is correlated with cardiomyopathy, and hyperglycemia can be associated with longevity[32].

Moreover, compared to mice on a control diet, experiments with high-calorie diets show that the mice gained significant weight[21,28], developed glucose intolerance[154], experienced lipid abnormalities [23], and had a decreased lifespan[28]. Dietary factors that cause oxidative stress lower the survival rate and speed up the ageing process[155,156], and chronic inflammation[45,82], mitochondrial dysfunction[146].

Furthermore, increased caloric intake also modify the diversity of gut microbiota, promoting metabolic dysfunction and shortening the average lifespan[60,157]. Furthermore, HFD can cause hyperglycemia and insulin resistance and decline survival[45,84,125].

The health and longevity of the infants can significantly impacted by maternal nutrition. Increased intake of calories, fat, and sugar in the mother's diet is connected with metabolic challenges that raise the risk of obesity development in both the mother and her progeny[30,60,63,123]. Pregnancy-related high-calorie food consumption and maternal obesity are also associated with progeny obesity[3,40] and cardiovascular disease later in life [158].

Although studies indicated a link between HFD and HSD in individual longevity, it is unknown whether the maternal diet influences the longevity of progeny. Thus, the role of maternal HFD and HSD on the progeny's lifespan was investigated in the present investigation. Male progeny of HFDm mothers showed a reduced median lifespan of 50 days in males compared with CDm, with 57 days, evidencing a robust negative effect of maternal HFDm on male longevity (Figure 17). Such a lifespan reduction

corresponds to the observations in complex organisms, where high-calorie diets reduce longevity and increase the susceptibility to metabolic and oxidative stress[29,30,50]. In contrast, HSDm did not significantly affect the lifespan of either male or female progeny, with median lifespans similar to CDm. In addition, PCA distributions were only evident in day 50 males of HFDm, and once the age factor was removed, the diet effect was perceptible in male progeny but not females (Figure 19). This means that, even if high-sugar diets affect metabolic and physiological parameters, the influence on longevity may be weaker or proceed along different lines than with high-fat diets.

Maternal obesity has been associated with the damaging effect on immunity and inflammation, in combination with either an HFD or HSD diet, is attributed to raising oxidative stress and gut microbiota changes that further alter fetal programming for metabolic disorders. These factors may influence fetal development and predispose offspring to metabolic disorders. The processes might include developmental dysfunction and epigenetic changes that lead to metabolic disorders persisting across generations and threaten the healthspan of the progeny[33,34,39].

Skoruoa et al. (2008) demonstrated that dietary ingredients can impact individually longevity and supply a practical basis for scrutinising the underlying genetic tools that connect typical nutrients with essential facets of public fitness and longevity[125]. The sex-specific impact of maternal diet on the lifespan of the progeny warns that gender variations should be taken into account in metabolic studies. Overall, the data suggest that male lifespans are more easily compromised under maternal HFD conditions, primarily through the loss of glucose homeostasis and elevation of metabolic dysfunction through ageing, compared with females.

HSD can boost insulin opposition and defecting functions of ovaries in the female *Drosophila*; nevertheless, descendants' survival was intact compared to CD[43]. HFD can lower the development and antioxidant capability, inducing lipophagy, hypertrophied lipid droplets, and apoptosis, which results in a decline in survival [156]. Both premature embryos and oocytes of HFD-fed mice have diminished telomerase and telomerase reverse transcriptase activity[70]. The remarkable effects of prenatal BMI in promoting molecular longevity are maintained by the association between short telomere lengths in the placental and cord blood telomeres of the offspring and gestational maternal BMI [69].

In conclusion, the present investigation indicated that maternal obesity can influence the longevity of the offspring in a sex-specific manner and revealed the crucial role of dietary sources. Although previous studies demonstrated links between diet and longevity for individuals, the present study represents the first evidence that the maternal diet also could determine longevity progeny. Further preclinical animal research should focus on the molecular reason behind the investigated pathology in longevity.

## 4.5 The role of the maternal diet in the Immune response

### 4.5.1 The role of Plasmatocytes in maternal obesity

Studies revealed that the immune cell density could be influenced by inflammation and response to pathogenice infections in mammals[1,2]. Plasmatocytes are the essential systemic protector cells of the fly immune system and have significant effects on immune regulation and metabolic processes, especially phagocytosis[96,114,138]. Previous studies have shown that immune cell numbers and morphology are influenced by metabolic diseases such as obesity[2,159]. Therefore, their density and functional alterations can be determinants of general health and life span. In this study, the plasmatocyte counts of both female and male fruit flies were initially conducted on days 0, 10, and 50 to investigate the relationship between a high-calorie maternal diet and possible inflammation and determine the role of the immune system. The present findings showed that plasmatocyte counts did not cause a difference between the groups for both sexes at any time point, which indicated that maternal HFD and HSD did not affect the cell density of plasmatocytes in *Drosophila* (Figure 27).

Third of the genes are known to affect organismal health and survival, and as indicated in The Heat-map of positive regulation of the innate immune system, at day 50, in the HFDm male group, revealed the downregulation of genes linked to the immune system, especially NF-kB, MyD88, and Upd3, compared to CDm and HSDm (Figure 26). A previous studies demonstrated that obesity upregulates the activation of the MyD88 and NF-kB levels in gestational diabetes mellitus[160]. However, it is remain unclear whether maternal obesity has any impacts on the particular processes.

Although, the maternal impacts of NF-kB, MyD88, and Upd3 not illuminated yet, studies revealed their functions in immune system of individuals. For example, in *Drosophila*, MyD88 is one of the critical components in the immune response to

pathogens and has functional and structural functions similar to those of mammals[104]. MyD88 levels at the molecular level may be a determinant of general health and longevity in fruit flies[104,161]. Next, My88 levels are known to increase in the event of infection[159]. Moreover, long-term HFD and injections of saturated free fatty acid result in reduced inflammation and hypothalamus reactive gliosis in the astrocyte-specific Myd88 KO animals[162].

Upd3, a member of the unpaired family, activates immune response via the JAK/STAT pathway and can affect many cellular processes[96]. Upd3 can alter metabolic processes, primarily insulin signaling and fat body functions, and affect glucose homeostasis. Thus, it may be influential in determining the life span[163]. Although the Upd3 cytokine cannot reduce fat accumulation, genetically depleting macrophages or silencing Upd3 specifically on macrophages reduced JAK-STAT activity, restored insulin sensitivity, and extended the lifespan of *Drosophila*[96]. However, in the present investigation JAK-STAT activation was significantly lower solely in HSDm males in day 0 and remained steady for other groups (Figure 28).

NF-kB, also known as relish, is a highly active structure in the immune system response in fruit flies and is one of the most essential protectors against pathogens[83,164]. In the absence or deletion of Relish, organismal health is impaired, and lifespan may be reduced in flies[165,166]. However, uncontrolled expression of NF-kB and low-grade chronic inflammation may shorten lifespan in fruit flies[167]. For this reason, some studies have shown that NF-kB depletion could increase fly survival in inflammation-related processes. NF-kB mutations are exceptionally gullible to infection, and a mutant fly is probably killed by a single E. cloacae cell[168]. Next, HFD can reduce the relish signaling and lead to a drop in iNOS and NO levels downstream and an increase in MDA and the suppression of the NF-κB/NO signaling pathway, which mediates pro-oxidant and antioxidant targets for the regulation of oxidative stress, maybe the molecular mechanism behind it[156]. Moreover, depletion of NF-kB can raise the longevity and median ages of flies[166]. In addition, NF-κB inhibition in IPCs is adequate to improve survival under diverse lethal circumstances[165].

Thus, NF-kB, Upd3, My88 genes are essential defence components of the cellular immune system, its deficiency could affect longevity in individuals. But their roles in the maternal obesity illuminated yet. Thus, the longevity of hemocyte-specific depleted NF-kB, Upd3, My88 investigated in maternal obesity. The longevity assay revealed that,

on the one hand, the hemocyte-specific Upd3 deficient flies reduced mean survival and, and NF-kB deletion significantly worsened longevity. On the other hand, the longevity of hemocytes-specific My88 depletion was comparable. (Figure 29). These findings suggest that maternal obesity results in a blunted immune response in *Drosophila*. Likewise, a study in primates demonstrated that maternal obesity leads to a blunted immune system response and that NF-kB has a vital role in this[36].

In conclusion, the present investigation revealed that HFD can blunt the immune system response, especially in male progeny. Although the density of plasmatocytes was comparable, maternal HFD resulted in a decline in NF-kB, Upd3, and My88 levels. Moreover, plasmatocyte-specific depletion of NF-kB worsened the longevity of the progeny. Thus, the present study suggests that the source of maternal diet can be crucial in the determination of immune capability and longevity in *Drosophila*. Further experimental studies are needed to elucidate the underlying mechanism and to examine the complex relationships in mammals.

### 4.5.2 Survival and climbing performance following the sustained antibiotics treatment

The *Drosophila's* microbiota is vital in combating pathogens, and antibiotic exposure could affect locomotor health and lifespan[169]. Disturbances in microbiota significantly affect behaviour and general health, including the neurodegenerative models of *Drosophila*, highlighting their relevance to neurological and physiological health[101,170,171].

Maintenance of a homeostasis in microbiota, mediated partly by the response of immune response, predominantly through *Drosophila*'s antimicrobial peptides, is critical for a long and healthy lifespan. Moreover, it has been demonstrated that antibiotics have the dual impact of eliminating balance in gut flora and preventing infections[169]. Disrupting the microbiota balance brought about by antibiotics like streptomycin and penicillin cuts down the fruit fly *Drosophila*'s life span and reduces survival[169,172]. The detrimental effects of antibiotics in *Drosophila* are rendered worse by microbiota, which interferes with how nature and microbiota interact in predicting the host's state of health.

For instance, in *Drosophila*, the recovery from a highly severe infection, such as sepsis, can have long-term health effects conserved in humans, whereby the triggering of immune responses after infection ultimately affects longevity behaviour[172]. Such

knowledge of antibiotic tolerance of *E. coli* found in the gut of *Drosophila* points to the astounding difficulties in handling bacterial infections without disturbing the balance of abilities in the microbiota that are critical for host health and longevity[169].

The peak concentration of streptomycin can remarkably diminish the longevity of all development steps and the adult longevity of bugs when corresponding to the control diet without streptomycin[173]. On the other hand, an antibiotic, namely minocycline, can boost the life span of *Drosophila* but also improve their longevity and climbing by likely modulating the gut microbiota beneficially[124]. Moreover, maintaining a normal gut microbiota is implications in the climbing and longevity of *Drosophila*. Thus, in the present investigation, the effects of chronic antibiotic treatment were tested in the progeny of *Drosophila*. While antibiotic treatment in males improved climbing ability significantly in both HFDm and HSDm groups, and survival in HFDm (Figure 30). This finding would imply that altering microbial exposure through antibiotics could provide a respite to physiological and metabolic challenges emanating from maternal nutritional exposures. Improved climbing suggests better neuromuscular ability and, in all probability, better metabolic health in antibiotic-treated conditions.

Thus, findings so far underscore the potential of microbiota manipulation or immune boost as interventions to counteract the detrimental effects of maternal obesity and high-caloric diets on the health trajectory of the progeny[60,124,169,171,174].

In summary, chronic antibiotic treatment mitigated some adverse effects on HFDm, particularly longevity and locomotor function, which offers potential for future health interventions. These results demonstrate the improved longevity and climbing function in male flies of HFDm as an impact of chronic antibiotic administration. Moreover, the present investigation revealed the crucial role of microbiota in developmental and gerontological perspectives. Further studies should focus in modulatory role of microbiota in maternal diet in determination of survival and locomotor ability.

### 4.5.3 Bacterial Load and Survival post-bacterial Infection

Besides their immediate metabolic implications, maternal obesity and diet habits throughout pregnancy are essential in modifying the progeny's immune-related response to pathogens[47,78]. This outcome only served to accentuate the long-term impact of maternal health on the progeny, beginning in development and occasionally carrying into maturity[3,67,73]. Parents infuse in their descendants by equipping them for

defence against parasites and pathogens that are solely faced parentally, a marvel comprehended immune priming that can be handed paternally and maternally to the descendants, hence raising the survival of progeny challenged to the identical pathogen[175].

Maternal fat immunomodulates the progeny and triggers low-grade inflammation that can be determined by increased amounts of C-reactive protein, interleukin 6, and tumour necrosis factor-alpha[22,48]. The developing fetus undergoes exposure to an inflammatory environment prior to birth because these inflammatory agents also cross the placenta[22,143]. The immunological response that arises later in life may be subject to this prenatal exposure, which may predispose the fetal immune cells to a proinflammatory phenotype[22].

To comprehend host-microbe interchanges, *Escherichia coli (E. coli)* can be an influential implement in flies as they do not inherently carry the bacteria densely in standard conditions[176]. On the other hand, HSD consumption in rodents results in quite an elevated quantity of *E. coli* in gaita samples[79].

To confirm whether the maternal HFD blunts the immune system, the flies challenged with *E. coli* injections. Although the bacterial load was relatively higher in the day 1 HFDm for both half and 2 million bacteria per fly, surprisingly, in comparison to the CDm group, HFDm had no discernible impact on the bacterium load (Figure 31) and survival post-infection (Figure 32). Opposed to the bacterial infection model, the mother's diet composition in this model appears to exert no effect on the bacterial load when compared to other groups. This suggests that the variations to the mother's diet may have little effect on the host's early pathogen clearance mechanisms. Accordingly, the early immune response to bacterial infections may be shaped by immunological regulation and microbial connection, amongst other variables of excessive dominance.

These effects can be further potentiated by high-fat diets during pregnancy, likely by the combined consequences of maternal obesity and worsened gut microbiota composition and function [60,169]. Hence, it permits the maternal predisposition for gut microbiome dysbiosis to generate further metabolites, like SCFAs, which would impact fetal immune programming by modulating immune cell differentiation and function[34,177]. The heritage of the maternal microbiome may be moderated by epigenetic mechanisms and microRNA expression, which can be engraved on the gametes by either parent without genetic changes [175].

As an illustration, high-sugar maternal diets cause inflammation, oxidative stress, and hyperglycemia, which may cause reprogramming of fetal immune cell function and impaired growth of immunological memory in that progeny. In the present investigation, the unanticipated level of resistance indicates that maternal HFD has unique roles in survival and the inflammatory response to bacterial infections.

The progeny born from an obese mother could end up born with the result of a blunted immune response that operates poorly, making them vulnerable to infections from an early age and more liable to develop chronic inflammation-related illnesses[36]. The latter implies that the impacts of maternal obesity extend beyond their effects on the development and function of fetal immune cells[36,48,61].

In summary, the survival of male progeny was comparable post-bacterial infection, and did not present any discernible impact on pathogen clearance. The current findings indicate the complexity by which maternal nutrition can set up progeny resilience to bacterial stress and contribute to the rapidly expanding field of developmental programming impacts on immunological and metabolic health.

## Chapter 5: Conclusion

The prevalence of obese individuals is rising worldwide, and considerable proof implies that progeny born to obese and overweight ascendants are affected by damaging health influences that dramatically diminish the fitness during ageing[30].

Maternal obesity can blunt immune response[36], accelerate epigenetic ageing[33], trim telomeres[69,70], grow the susceptibility gut dysbiosis[178], cancer[58], diabetes and heart illness[3], and thus significantly affect progeny fitness in the long term[73], which can result in diminished life expectancy. Nevertheless, hitherto, it has not been comprehended how maternal obesity longitudinally affects health, including physiologic, metabolic, and genetic regulations, and its relationship with longevity. Further, the role of the innate immune system in the observed pathology remains unclear. Moreover, there is a gap in using both HFD and HSD in the same experimental design for insight into how dietary sources influence health differently regarding both male and female progeny. Therefore, in this experimental study using fruit flies, I investigated whether maternal HSD or HFD could affect progeny's longevity and health by examining genetic, physiological, axonal and immune regulations in both male and female progeny.

In female progeny, although most parameters remained stable compared to the CD group, a decline in body weight due to maternal diet was detected on day 50, which corresponded to the CDm group, and genetic regulations were noticed forming on day 10. Moreover, the effects of maternal obesity on male progeny were more influential in the long term, while HFD and HSD-induced maternal diets raised the number of differently expressed genes and adversely affected the parameters of metabolism, climbing ability, and weight gain. In contrast, axonal degeneration, longevity and immune system-related parameters deteriorated only in the HFDm group (Figure 33). These results show that both maternal dietary sources may have a sex-specific effect on the development of diseases. Moreover, the current study offers firsthand scientific proof of the long-term health effects of maternal obesity on offspring. In prospective investigations, it will be vital to prove this hypothesis by a driver line targeting only plasmatocytes, using genetic knock-out or knock-down techniques. Next, the role of NF-kB can be tested by knock-in lines to examine whether the pathophysiology is healing. In addition, including epigenetics and microbiomes in sexual dimorphism could be a concern for approaching studies.

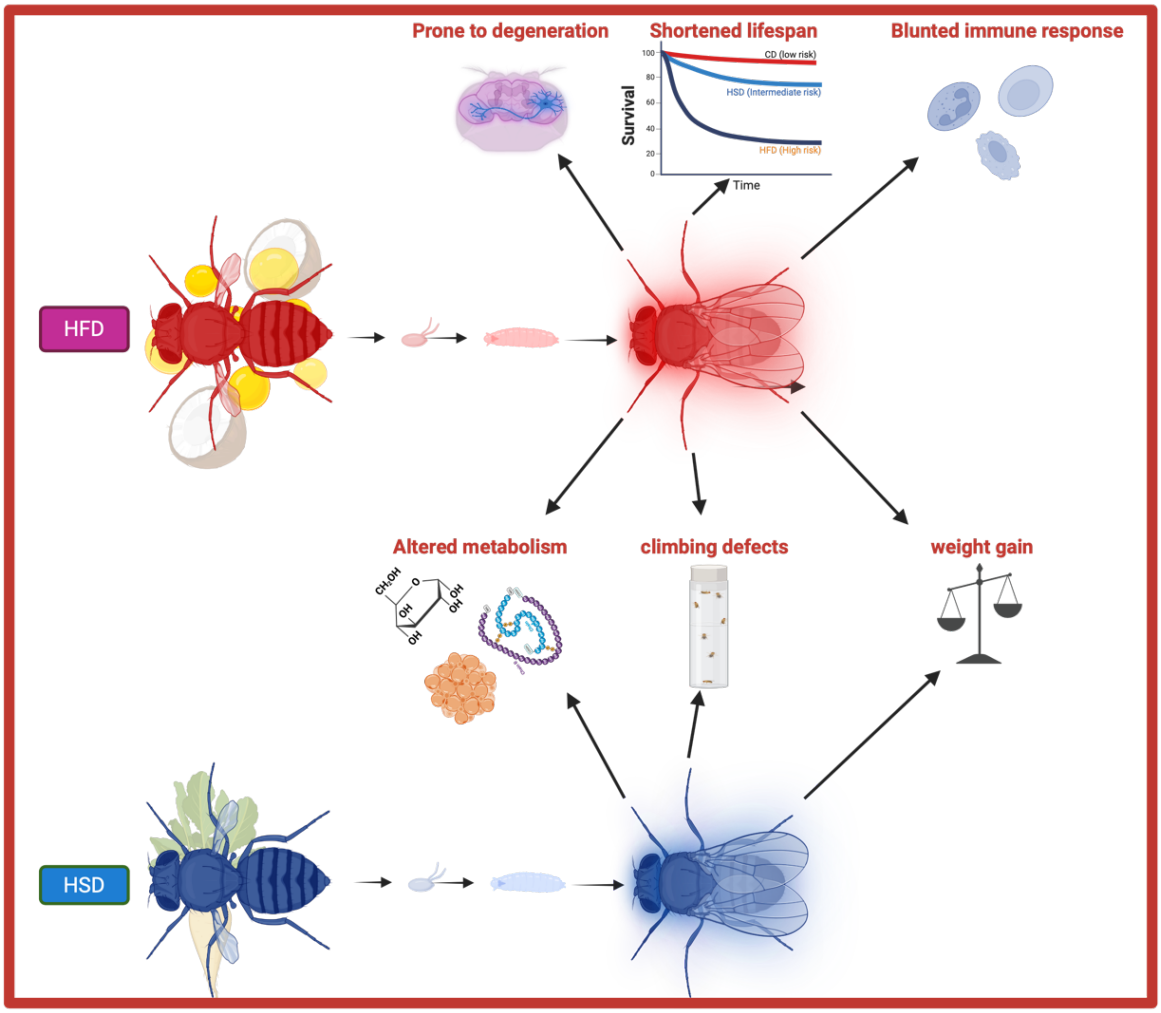


**Figure 33: Impact of the maternal diet on male progeny of the *Drosophila.*** The figure summarizes the effects of maternal high-fat (HFD) and high-sugar diets (HSD) on male progeny. Both maternal HFD and HSD affected metabolism, body weight, and climbing performance. Only maternal HFD increased axonal degeneration and diminished longevity.

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