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Drosophila Melanogaster As A Model For Diabetes

Diyabet Modeli Olarak Drosophila Melanogaster

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ABSTRACT

Diabetes is a serious global concern, affecting a huge proportion of the world's population. Recently, scientists have used *D. melanogaster* as a model organism for metabolic diseases such as type 2 diabetes, type 1 diabetes and obesity to study insulin signaling and metabolic pathways. The insulin pathway and glucose control of sugar metabolism human and *D. melanogaster* have been found to be preserved despite the physiological differences. Therefore, *D. melanogaster* is an optimal model for establishing a new perspective to human metabolic diseases. *D. melanogaster* is a more advantageous model organism compared to other invertebrate animal models due to its 75% similarity to the mammalian genome in modeling diseases seen in humans diseases. *D. melanogaster* diabetic disease models will help identify pathways associated with insulin resistance and the absence of additional genes related to type 2 diabetes in the future. In the near future, the high gene similarity between *D. melanogaster* and the mammal and the convenience of *D. melanogaster* in the laboratory will allow an understanding of the mechanisms underlying various metabolic diseases. In this study, recent reports which show how *D. melanogaster* can be one of the best model organisms for diabetes modeling using different manipulation methods have been compiled to better address the utility of the *D. melanogaster* type 2 diabetes model in medical research.

Keywords: Diabetes, *Drosophila melanogaster*, high fat diet, high sugar diet, mutation, model organism.

ÖZET

Diyabet, dünya nüfusunun büyük bir bölümünü etkileyen ciddi bir küresel sorundur. Son zamanlarda, bilim adamları *D. melanogaster*'i insülin sinyalini ve metabolik yolları incelemek için tip 2 diyabet, tip 1 diyabet ve obezite gibi metabolik hastalıklar için model organizma olarak kullanmışlardır. İnsan şeker metabolizması ve D. melanogasterin insülin yolu ve glikoz kontrolünün fizyolojik farklılıklara rağmen korunduğu bulunmuştur. Bu nedenle, *D. melanogaster*, insan metabolik hastalıklarına yeni bir bakış açısı oluşturmak için optimal bir modeldir. *D. melanogaster*, insanlarda görülen hastalıkları modellemede memeli genomuna %75 benzerliği nedeniyle diğer omurgasız hayvan modellerine kıyasla daha avantajlı bir model organizmadır. *D. melanogaster* diyabetik hastalık modelleri, insülin direnci ile ilişkili yolları ve gelecekte tip 2 diyabet ile ilgili ek genleri belirlemeye yardımcı olacaktır. Yakın gelecekte, *D. melanogaster* ile memeli arasındaki yüksek gen benzerliği ve laboratuvarda *D. melanogaster*'in kolay kullanımı, çeşitli metabolik hastalıkların altında yatan mekanizmaların anlaşılmasına olanak sağlayacaktır. Bu çalışmada, *D. melanogaster*'in, farklı manipülasyon yöntemleri kullanarak diyabet modellemesi için en iyi model organizmalardan biri olabileceğini gösteren son raporlar, *D. melanogaster* tip 2 diyabet modelinin tıbbi araştırmalardaki faydasını daha iyi ele almak için derlenmiştir.

Anahtar kelimeler: Diyabet, *Drosophila melanogaster*, yüksek yağ diyeti, yüksek şeker diyeti, mutasyon, model organizma.

INTRODUCTION

Diabetes is a serious global concern, affecting a huge proportion of the world's population. Diabetes can be classified as type 1 and type 2, with type 2 diabetes (T2D) taking up a substantial capacity of the total diabetes cases. T2D is highly associated with obesity[1]. Impaired glucose tolerance which makes for pre-diabetes is a high risk factor. Up to 70% of individuals with pre-diabetes eventually develop T2D[2]. Insulin resistance (IR) is associated with pre-diabetes. Therefore, understanding IR mechanisms is necessary for preventing T2D and developing specific treatment strategies. However, it is not too easy, in the clinical scenario, to study such aspects in depth.

Scientists have developed model systems in which diabetes can be controlled at a higher level than in humans. Moreover, experimental designs can be established with high precision and reproducibility as well as being with appropriate genetic uniformity in highly controlled environments. The insulin pathway and glucose control are common in mammals and invertebrates. Therefore, the principles laid out in these organisms can be applied and extrapolated on a general scale. Among these experimental animals, *D. melanogaster* is a good example to such an organism in that it posesses similarity between to the mammalian genome.

The *D. melanogaster* genome contains conserved, NAD-dependent histone deacetylase sirtuin (SirT), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), members of the class O of forkhead box transcription factors (FOXO), target of rapamycin (TOR), serine/threonine protein kinase B (Akt) and nuclear receptors metabolism regulators. These are significantly similar to mammals[3]. *D. melanogaster*'s insulin-like peptides (DILPs) are involved in glucose homeostasis. DILPs and adipokinetic hormone (AKH), the glucagon analogue, help maintain circulating glucose levels. In the type 1 diabetes model, the loss of insulin-producing cells causes the in hemolymph sugar to increase[4]. Conversely, the loss of corpora cardiaca reduces the amount of hemolymph sugar by generating AKH[5].

D. melanogaster is suitable for modeling defects in experimental and genetic manipulation due to the small number of chromosomes compared to rodent models. In addition, the fact that it is easily grown, stored and produced in a laboratory environment, as well as producing a large number of embryos in a short time makes *D. melanogaster* a suitable model organism in the modeling, diagnosis and treatment of human diseases, especially metabolic diseases such as T2D.

In this study, the similarity between *D. melanogaster* and mammalian organisms in terms of genomic and organ systematics of diabetes, which increases in numbers by the day and has been rapidly becoming a pandemic, have been taken into consideration. It has also been made to demonstrate that *D. melanogaster* can be easily manipulated due to its easy growth, mating and storage in the laboratory, as well as its low chromosome numbers and easy manipulation. Recent reports which show how *D. melanogaster* can be one of the best model organisms for diabetes modeling using different manipulation methods have been compiled to better address the utility of the *D. melanogaster* T2D model in medical research.

Drosophila Melanogaster As A Model Organism

D. melanogaster is used as a model organism in the study of many biological processes from genetics and inheritance to learning and

behavior. The mechanisms and signaling pathways that control development and survival are preserved in the invisible animal kingdom. Therefore, using *D. melanogaster* as the model organism is a good option, and the models shown in *D. melanogaster* can then be adapted to other model organisms[6]. In recent studies, *D. melanogaster* has begun to be used extensively in modeling metabolic diseases such as obesity and diabetes[7].

Due to the fact that *D. melanogaster* has few chromosomes and a small genome size, it is easier to perform gene manipulation in various ways than more developed organisms. Because of this feature, it is a preferred model organism for its molecular mechanisms. *D. melanogaster* has several advantages over other invertebrate models, including Caenorhabditis elegans and Saccharomyces cerevesiae. *D. melanogaster* is preferred over these two invertebrate models because of its very similar organ system to that of mammals. *D. melanogaster* has a short life cycle, ease of genetic manipulation, sequenced genome, and numerous other technical possibilities which help cover all possible genetic experiments. Interestingly, most of the human genes associated with diseases have homologues in *D. melanogaster*, which allows the adaptation of suitable models based on the endogenous genes of *D. melanogaster*[8,9]. This intense genetic similarity means that the underlying mechanisms associated with, or underlying diseases, are preserved throughout evolution. In some cases, *D. melanogaster* can also serve as an "*in vivo* test tube" in gene function testing[10,11].

The use of *D. melanogaster* in glucose homeostasis mechanisms, and basic physiological and cellular processes facilitates interpretation of the obtained results. This is also the case when a human disease is caused by a single gene or protein malfunction. *D. melanogaster* provides a unique opportunity both to study development and to uncover critical new strategies in better defining the critical signaling pathways which will enlighten the underlying specific causes of the human disease in question.

High Conformity with the Human Genome

The *D. melanogaster* genome has been completely sequenced and explained. When the genome sequence was examined, it was seen that more than 14,000 genes were encoded on four chromosomes, of which only three carry the majority of the genome. It is known that approximately 75% of the genes related to diseases in humans have functional orthologs in *D. melanogaster*[9,12] (Table 1).

Table 1. *The D. melanogaster metabolic mutant genes and their human orthologs.*

***D. melanogaster* metabolic mutant gene**	**Human Ortholog(s)**
dilp 2,3,5	Insulin/IGFs
chico	IRS1-4
dFOXO	FOXO1, 3a, 4
AKH [A]	Glucagon
Brummer	ATGL
Adipose	WD40 and tetratricopeptide repeats
dTOR	TOR
4E-BP	4E-BP
dSREBP	SREBP-1a/SREBP-2

The overall identity at the nucleotide level or protein sequence between *D. melanogaster* and the mammal is generally about 40% between homologs; however, it is known that it may be 80% to 90% or higher in protected functional areas.

Thomas Hunt Morgan was the first to realize the mapping potential of *D. melanogaster*'s chromosomes. It is the best genetically known eukaryotic organism today. *D. melanogaster*, whose entire DNA sequence was completed in

2001, has 14,000 genes and 165 million base pairs.

Similar Organ Systems Between Humans and D. melanogaster

In recent studies, it has been found that metabolic pathways are similar between mammals and *D. melanogaster*, with the discovery of similar organs and enzymes that regulate metabolism[13–16].

Most of the similar organ systems that control food intake, storage and metabolism in humans are also present in *D. melanogaster*. *D. melanogaster* contains a midgut, which is the equivalent of stomach and intestine in humans. As an analogous organ of adipose tissue, the fat body metabolizes nutrients and stores large amounts of glycogen and lipids. It contains oenocytes as a functional analogue of the liver. Oenocytes accumulate lipids in the starvation state and function as hepatocytes in lipid processing[17,18]. In addition, individual cell clusters preserve *D. melanogaster*'s carbohydrate homeostasis, similar to pancreatic a and p cells[19,20] (Figure 1).

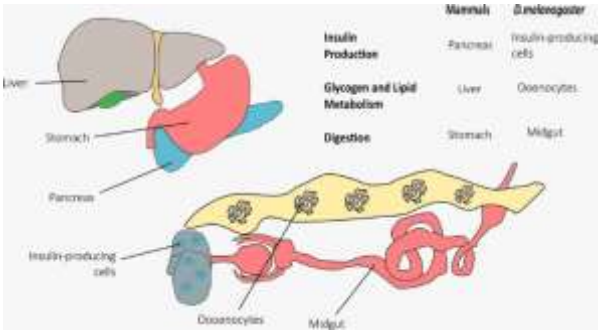


Figure 1. *Similar organ systems in humans and D. melanogaster.*

An important difference between mammalian and *D. melanogaster* metabolism is that *D. melanogaster* cannot synthesize cholesterol[21]. Nevertheless, *D. melanogaster* can still be used in understanding the cholesterol metabolism.

D. Melanogaster In The Laboratory Setting

D. melanogaster has many advantageous properties that allow scientists to easily research in their work:

- It has a short life cycle,
- It is easy to cultivate and maintain,
- They have few chromosomes and a small genome size but contain giant salivary gland chromosomes known as polytene chromosomes.

Figure 2 gives an example of how the genetic material of *D. melanogaster* can be observed at the laboratory under a light microscope.

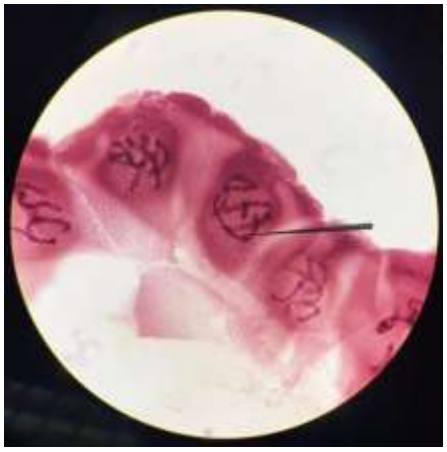
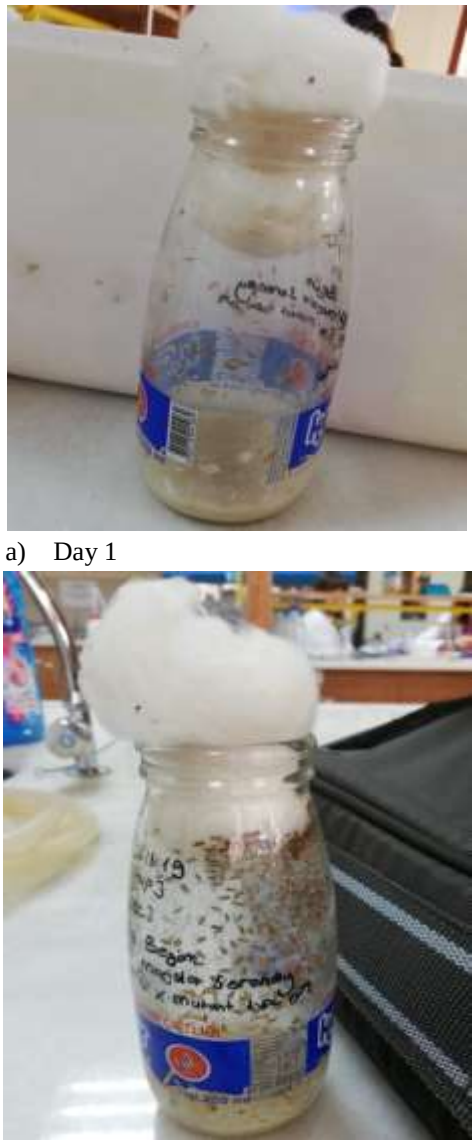


Figure 2. *Light microscope image (40X) of the polytene chromosome removed from the salivary glands of D. melanogaster larva and stained with hematoxylin.*

D. melanogaster has a very rapid life cycle. The female *D. melanogaster*, about 3 mm long and lays 750 to 1500 eggs during a full lifespan. The life cycle of *D. melanogaster* takes only 12 days to complete at ambient temperature (25 ° C). After the egg (only half a millimeter long) is fertilized, the embryo emerges within ~ 24 hours (Figure 3). *D. melanogaster* can be considered as an all-in-one organism combination. Within *D. melanogaster*, each individual organism in this sense – embryo, larva, pupa and adult – will have certain advantages. The embryo is often used in basic developmental studies that examine pattern formation, cell fate

determination, organogenesis, neuronal development, and axonal finding. The larva, particularly the third instar larva, are used as a model for studying developmental processes, physiological processes, and simple behavior.



a) Day 1

b) Day 7

Figure 3. *D. melanogaster breeding and propagation, in vitro.*

Future adult structures of *D. melanogaster* are found in larvae as imaginary discs consisting mainly of undifferentiated epithelium. Adult *D. melanogaster* consists of the occurrence of morphological changes starting from the late third larval stage and continuing through the pupal stage (Figure 4). Examination of the molecular and genetic mechanisms underlying the imaginary disc development processes in the pulp makes the pupa a suitable model to investigate specific developmental processes[22].

Figure 4. *L3 (3rd stage larva) D. melanogaster microscope image (40X).*

Even though a small creature, *D. melanogaster* provides researchers the ability to easily navigate within basic metabolic functions by making use of assays such as mitochondrial activity measurements, ATP analyses, lipid metabolic profiling, insulin tolerance tests, the stored mother lipid form, triacylglycerol (TAG) and circulating sugar levels. In addition to these, it is possible to perform sensitive tests for specific metabolic responses that cannot be performed in more complex vertebrate systems, such as the Green Fluorescent Protein (GFP) test for membrane-associated phosphatidylinositol 3 (PIP3) in intact tissues in *D. melanogaster*[23].

D. Melanogaster As A Genetic Model For Diabetes

In developed organisms, conserved insulin/IGF pathways are central to metabolism and growth. In mammals, the primary role of IGFs is to regulate growth,

whereas the primary role of insulin is glucose homeostasis.

There is a point where these two activities converge in the insulin/IGF pathway. The seven insulin-like peptides (DILP1-7), whose functions are not yet well-defined, act via an insulin-like receptor (InR)[24,25]. InR is related to the chain reactions involving certain genes, taking place in the insulin/IGF pathway on an intracellular level. Such genes include the insulin receptor substrate (IRS) Chico, the insulin signal antagonist PTEN, PI3K, PKB/Akt kinase, and the single FOXO ortholog dFOXO[26].

Expression of dilp3 and dilp5 from three dilp genes (dilp2, dilp3 and dilp5) expressed by neurosecretory cell clusters in the brain under normal nutritional conditions is an insulin-producing in cells (IPCs), low carbohydrate diets[24,25,27]. This shows that *D. melanogaster* gives a similar response to that of dilp expression in humans at different food levels[25,28]. Moreover, the loss of IPC shows an increase in circulating glucose and trehalose and lipid storage[4,29]. IPCs appear to function like pancreatic β-cells as they secrete DILPs into the circulatory system to maintain properly circulating sugar levels, although it is not yet known whether DILP secretion is regulated by IPCs[4].

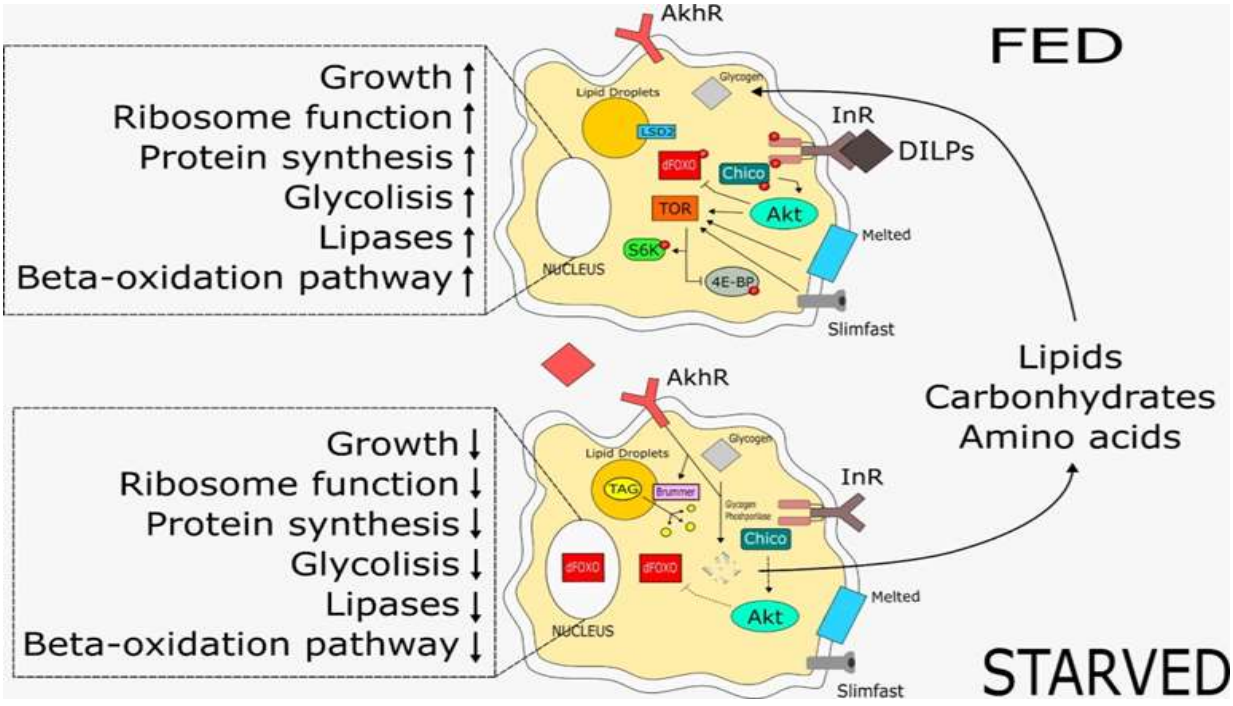
In *D. melanogaster*, the adipokinetic hormone (ACH), which acts similarly to the pancreatic α-cells that secrete glucagon in mammals, balances the amount of insulin: activating glycogen phosphorylase, reducing fat body glycogen, increasing the amount of sugar in the circulation. The inhibition of AKH function does not seriously affect glucose or lipid levels[5,19,30,31]. ATP-sensitive potassium (K_{ATP}) channels act similar to pancreatic a cells in glucagon secretion[5].

These mechanisms help us understand that insulin and glucagon are preserved through evolution. Hence, it is easily possible to comment that *D. melanogaster* is a valid model for the studies on the human metabolism related to glucose levels and diabetes control (Figure 5).

5. Inducing Diabetes In *D. Melanogaster*

T2D can be created by various manipulations in *D. melanogaster* (Table 2). These are: i) diets that cause obesity, metabolic imbalance and hyperglycemia and different lifestyles, and ii) function on the ILP gene It is mutations in the insulin pathway that cause loss[32-36].

Figure 5. *Metabolic signaling pathways in the starved and fed in D. melanogaster.*



Inducing Diabetes	
1. Dietary manipulation a) High fatty diet - Coconut oil - Dshoulder oil - Soybean - Palm oil b) High sugar diet - Glucose - Fructose - Sucrose	It has been observed that the amount of triglycerides increases in *D. melanogaster* larvae subjected to high fat diet, causes cardiac lipid accumulation and insulin-TOR signaling is inhibited[32,37,38]. *D. melanogaster*, subjected to a high sugar diet, increased hemolymph sugar, decreased sensiti-vity to insulin in peripheral tissues, smaller than those fed with a normal diet[39], increased fat accumulation in the body and decreased expression of FOXO target genes are observed results[33].
2. Genetic manipulation	Effects of genetic manipulations on *D. melanogaster* metabolism manifest as imbalances in fat body morphology, increased TAG levels and increased sugar content in hemolymph[32,40–45].

Table 2. *Diabetes induction in D. melanogaster.*

Dietary Manipulation

Diets containing nutrients that can be increased or decreased in *D. melanogaster* show their effect on conditions such as fertility, life span, sugar and fat accumulation, weight change, insulin resistance change and aging in *D. melanogaster*[33,46,47] (Figure 6). As in mammals[48], due to overfeeding, *D. melanogaster* reduces the insulin response secondary to increased insulin-like peptides (ILPs) in the circulation[46]. However, this increase in insulin signal plus hyperglycemia leads to an increase in free fatty acids with inappropriate lipolysis and the formation of insulin resistance, especially in body fat[49]. In addition, high fat diet (HFD) contributes to heart dysfunction[32]. Although dietary manipulation diet regimens are chemically semi-defined at best, it is of great convenience to use them in research to induce and develop diabetic conditions similar to T2D in *D. melanogaster*[47]. Also, these studies are based on environmental factors, gender, genetic factors, species, age etc. varies depending on the study, and all of these affect the results of the studies.

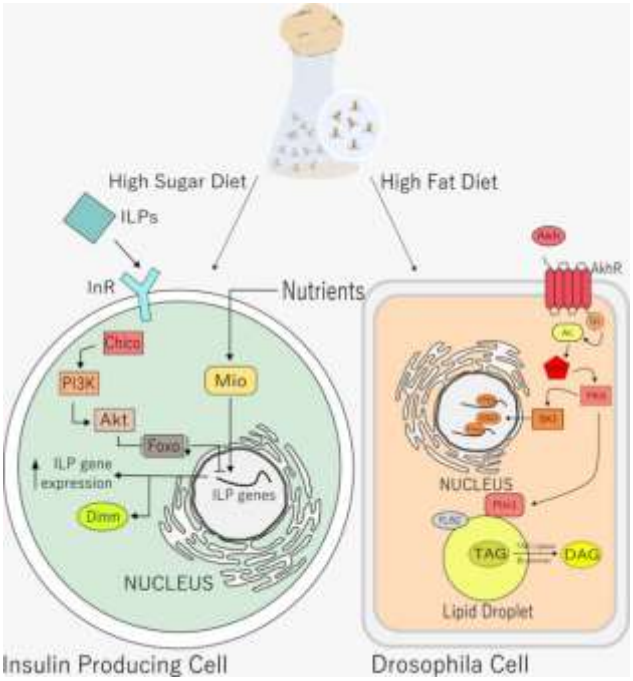


Figure 6. *Changes in metabolism as a result of a high fat and sugar diet in D. melanogaster.*

High fat diet (HFD)

D. melanogaster is also the most used coconut oil supplement to induce obesity and diabetes due to a high fat diet[32,49–51]. However, together with this, hydrogenated soybean and palm oil known as lard and Crisco are used[33,52,53].

While HFD causes lipid accumulation in *D. melanogaster*, it also responds similarly to mammalian organisms. Similar responses are increased triglyceride (TG) and changes in insulin/glucose homeostasis. Such metabolic and cardiotoxic phenotypes induced by HFD are blocked by inhibiting the insulin-TOR signaling pathway. Moreover, reducing insulin-TOR activity (expressing TSC1-2, 4EBP or FOXO) or increasing lipase

expression is sufficient to treat fat accumulation and dysfunction caused by HFD[32].

In mammals, increased TG levels are an important risk factor for insulin resistance[37,38]. *D. melanogaster*s fed with HFD were found to exhibit increased triglyceride (TG) level and changes in insulin/glucose homeostasis, similar to mammalian responses.

Since high TG levels are associated with impairments in lipid and glucose homeostasis, mitochondrial function and other processes, such levels are an important marker for phenotypes caused by HFD[37,38,54–56] and all cause high lipid accumulation and cardiac complications.

Consequently, the excess dietary fat consumption which is associated with TG accumulation can induce a number of associated diseases such as T2D, liver and cardiovascular dysfunctions and colon cancer[54–56].

High sugar diet (HSD)

In *D. melanogaster*, the most commonly used for high sugar diet (HSD) is high sucrose diets[27,33,39,57–61], but diets with high glucose and high fructose are also used[62].

The development of hyperglycemia is typically modeled in D. melanogasester larvae[33,39,63]. It is marked by an increase in trehalose which is the primary sugar circulating in the organism[33]. Larvae of *D. melanogaster* are significantly smaller in size when compared with those assigned to the control group[39].

An HSD also causes increased fat, and is insensitive to the action of insulin. the expected As expected, by HSD, the FOXO target gene expressions are also induced along with the preceding insulin signaling[33].

D. melanogaster larvae put on HSD, exhibit severe hyperglycemia. This is similar to that of insulin-resistant *D. melanogaster* whose IPCs were inactivated[4,64]. Additional experiments reveal that HSD-fed larvae also exhibit peripheral insulin resistance[33]. A second larval diabetic model of *D. melanogaster* corroborates the study of Musselman et al. (2011)[33] in that it has also revealed peripheral insulin resistance as a result of HSD[39]. Pasco and Léopold (2012)'s study additionally suggest that forced secretion of dilps can overcome the effect of HSD[39].

Lipocalin neural lazarillo (NLase), associated with metabolic homeostasis, is an ortholog of vertebrate lipocalins such as lipocalin 2 and the retinol binding protein 4 (RBP4). With its activity, NLase mediates peripheral insulin resistance triggered by HSD[65,66].

Adult *D. melanogaster*s on HSD, have arrhythmias, gradually succeeding to fibrillation and systolic periods[63]. This is much resemblant to all phenotypes observed in the *D. melanogaster* larval model[33,63].

Genetic Manipulation (Causing Mutations)

In modeling type 1 diabetes and T2D resulting from resulting from loss of function in ILPs and in the rest of the pathway affecting insulin resistance, respectively, is caused by mutations which lead to faulty insulin signaling[42,43,67]. In order to model diabetes in *D. melanogaster* by mutational manipulations, it must have more than one ILP gene loss of function mutation. This is because the seven ILPs found in *D. melanogaster* are partially dysfunctional[29]. Conversely, mutations in InR, Dp110, and other components of the pathway are homozygous-lethal. This is the reason why heterozygous mutations or heteroallelic *D. melanogaster* are often preferred over

homozygous mutations when modeling diabetes[40,67].

The most common effects caused by mutations in the insulin pathway on *D. melanogaster* are listed below[32,40–45]:

- decreased fertility,
- reduced organism size and lifespan,
- imbalances in fat body morphology,
- impaired heart, retina, and brain physiology,
- increased TAG levels, sugar content in the hemolymph.

FUTURE PROSPECTS

D. melanogaster diabetic disease models will help identify pathways associated with insulin resistance and the absence of additional genes related to TD2 in the future. The use of high-throughput screening and pharmacological intervention studies in these *D. melanogaster* models can offer an opportunity for research in therapeutic interventions for humans.

In the near future, the high similarity of the gene and organ system between mammalian and *D. melanogaster*, together with the ease of study of *D. melanogaster*, will provide an understanding of the mechanisms underlying various metabolic and genetic diseases.

CONCLUSIONS

Recently, scientists have used *D. melanogaster* as a model organism for metabolic diseases such as T2D, type 1 diabetes, obesity to study insulin signaling and metabolic pathways. These pathways of sugar metabolism between human and *D. melanogaster* have been found to be preserved despite physiological to differences. Therefore, *D. melanogaster* is an optimal model for establishing a new perspective to human metabolic diseases. *D. melanogaster* model organization helps to discover additional genes and mechanisms of such genes, develop novel therapeutic approaches, and develop diagnostic and hormonal tests.

It is a very suitable model for various aspects of metabolic diseases. Dietary interventions in *D. melanogaster* such as HFD or HSD, mutations created on the insulin signal trigger T2D. As in mammals, due to overfeeding, *D. melanogaster* reduces the insulin response secondary to increased ILPs in the circulation. This causes insulin resistance to occur. These similar responses in mammals and *D. melanogaster* show that insulin signaling systems are protected from *D. melanogaster* to mammals, showing that *D. melanogaster* is a good model organism in modeling human metabolic diseases.

In conclusion, *D. melanogaster* is a more advantageous model organism compared to other invertebrate animal models due to its 75% similarity to the mammalian genome in modeling diseases seen in humans diseases diabetes, obesity, cancer, heart diseases etc. In addition, it is an easy organism in terms of growing, storing and working in a laboratory environment. *D. melanogaster* provides a great advantage for studies as it produces a large number of eggs and that the egg forms an embryo only 24 hours after fertilization. Having a small number of chromosomes facilitates gene transfer, gene analysis and mutation in the genes.

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ETHICAL STATEMENT

None.

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