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NUTRIGENOMICS STUDIES TO EXPLORE ANTIAGING: *Drosophila* APPROACH

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ABSTRACT

The field of Nutrigenomics has always been of interest to scientists as it integrates the field of nutrition and the "-omics". *Drosophila melanogaster* is the most suitable model for this study as appx. 60% of human diseases have homology in flies. Ageing is a complex mechanism that involves several pathways, and only a small fraction of these pathways has been studied in detail. There have been proofs that nutrition can extend or reduce life span. The epigenetic mechanisms involved have also been explained to an extent. Certain diseases that manifest due to ageing can potentially be delayed with the right nutrition and diet. Antioxidants and polyphenols help reduce the oxidative stress, and this study is important as oxidative stress is one of the main reasons of ageing. Many theories for ageing have been hypothesised and are under study. Nutrigenomics might be the break-through that changes the perception of "diets" and how beneficial the right diet can be on human health.

Keywords: Nutrigenomics, Anti-aging, *Drosophila*, Epigenetics, Telomerase activity, Gene expression

INTRODUCTION

Ageing and anti-ageing

Ageing is a phenomenon that is caused over a period of time due to various factors. Just like machinery are affected due to wear-and-tear, living organisms are affected too. The study of this process of ageing is called Gerontology. Ageing, although is a natural process, comes with its set of repercussions. They include several pathological conditions caused due to changes in the molecular level. To cope with such changes, there has been extensive research in search of anti-ageing remedies. The wear and tear caused can be due to oxidative stress, molecular damage, or even protein alteration due to molecular pathways.
Anti-ageing is an attempt to reverse the effects of ageing and is a huge marketing tool in the industry of fashion and wellness. The study of anti-ageing deals with three main processes. The first is compressed morbidity, second is decelerated ageing, and the third is arrested ageing. The study that deals with slowing down or even arresting these changes is "Biogerontology". The main goal of the above-mentioned processes is to prevent the onset of age-related ailments by understanding the molecular pathways involved **(Halldór Stefánsson, 2005)**.

Molecular damage

The molecular damage caused due to ageing is mainly due to accumulation of protein and nucleic acid metabolites which can result in oxidative damage. Recent studies also state that improper DNA repair mechanism which leads to accumulation of the damaged DNA also results in ageing. Every organ and tissue in the body undergo molecular changes due to which there is a decrease in functionality. There are various theories that have been proposed for the possible mechanism of aging, one of which is mitochondrial and free-radical theory of aging. The mtDNA present in mitochondria has a very high mutation rate and hence there is accumulation of damage in the mitochondria. So, the process of anaerobic respiration becomes faulty and hence it starts leaking ROS factors. As a result, there is increased oxidative stress on the cell. Telomere attrition also causes ageing **(Maynard Scott et. al., 2015).**
Another theory is ageing due to cell senescence. This is mainly caused due to DNA damage response activation, where p53 is stabilised, and p21 is activated (which is a Cdk inhibitor) by ATK signaling pathway which blocks the cell-cycle progression. The activation of DDR pathway is due to DNA lesions and ROS. Senescence is also caused by oncogenic Ras which causes an overexpression of Cdc6. So, there is abnormal DNA replication and results in Double Stranded Breaks and the DRA pathway is once again activated **(van Deursen J. M., 2014)**.

AGEING IN HUMANS

Aging in humans is a synergistic effect involving factors like free radical reaction, Maillard reaction, and glycation reaction. They all cause a very complex biochemical pathway that results in the manifestation of signs of aging **(Liochev, 2015).**
The Maillard reaction is the reaction between amino acids and reducing sugars. Glycation is a major reaction classified as a Maillard reaction where a sugar attaches to a protein or lipid. The reactions take place predominantly in cartilages. Advanced Glycation End product (AGE) is a pentosidine, and its accumulation increases with age. The markers were browning and fluorescence that were observed in the cartilage **(Verzijl ,2000)**. Maillard reactions also cause atherogenesis which is caused by collagen cross-linking. This cross-linking and degradation of elastic fibers cause rigidification of the vascular wall. Since the contractility is lost with age due to an increase in collagen cross-linking, Maillard reactions can also be the possible cause of end stage heart failure. The collagen cross-linking has been shown to cause ECM-aging which is directly reflected on the skin collagen **(Robert et al., 2013)**.
A disturbance in protein homeostasis due to protein misfolding, and other forms of protein damage. There is evidence of increased oxidation of proteins in the brain being linked to ageing. These kinds of covalent damages are generally irreversible. The chaperone molecules that repair the conformational damages are overwhelmed with increase in age and hence there is accumulation of protein damage. Hsp90 is

one such chaperone molecule that is upregulated under conditions of stress. Some chaperone molecules like Hsp70 also play a role in Chaperone-mediated Apoptosis (CMA).
Dysregulation in various cellular signaling pathways are one of the main reasons for disease progression due to ageing. The TOR signaling pathway that involves mTORC1 and mTORC2 are important for nutrient sensing, especially amino acids. The involvement of AKT and PTEN proteins in this pathway have been studied and there is proof that they are dysregulated with age **(McAuley, 2017).**

.PREVALENCE OF DISEASE ASSOCIATED WITH AGEING

The life span of the last couple of generations have increased and the population above the age of 85 is very high. Old people have been categorised as "young-old" (60 years and early 70s who are healthy and active for their age), "old" (70s and 80s, but with certain chronic conditions), and "oldest-old" who are in the palliative-care stage. Depending on the lifestyle, genetic, and epigenetic factors, the older population either face signs of normal aging or chronic conditions and somatic symptoms.
Some of the chronic conditions include cardiovascular diseases, hypertension, cancer, osteoarthritis, diabetes mellitus, osteoporosis, and multiple chronic conditions.
Cardiovascular diseases have been, and continue to be the leading cause of death amongst people belonging to the older age group. The CV conditions include atherosclerosis, vascular stiffness due to cartilage cross-linking, ischemic heart disease, and chronic heart failure.
After cardiovascular diseases, cancer is a close second as the leading cause of death in older people. However, after the age of 85, slow-growing tumors seem to be more prevalent so the number of deaths are lower. The life-expectancy purely depends on the functionality of the patient and the co-morbidities they present.
Hypertension, more specifically isolated systolic hypertension, is the most common chronic condition in people belonging to the "oldest-old" category. It contributes to atherosclerosis.
Osteoarthritis is the second most common chronic condition. Among people who are over 85 years of age, more than 50% of the population are diagnosed with osteoarthritis. Due to hormonal imbalance and increased bone resorption after menopause, osteoarthritis is more common in women than in men. A risk factor is obesity, and as the age progresses chances of hip and knee arthritis are very high.
The chances of being diagnosed with diabetes mellitus increases with age, and weight. At 85, it remains the most common contributor to cardiovascular diseases. It is also a contributing factor to peripheral vascular diseases, diabetic foot, and consequently amputations.
Osteoporosis is the decrease/loss of bone density with age. In women, the prevalence of fractures increases after 65 years. While in men, it is after 85 years of age. This is due to decreased bone mineralisation **(Jaul Efraim and Barron Jeremy, 2017).**

NUTRIGENOMICS

Nutrigenomics deals with the study of the effect of food on genes. It deals with already known interaction between food and genes and the effect it can impose. For example, in patients diagnosed with Phenylketonuria, foods rich in phenylalanine are avoided as it causes hemolytic effects (fava beans). Obesity is now considered a pandemic by WHO, and nutrigenomics will help in getting to the root of all causes. As a result of obesity, conditions like CHF, hypertension, and type-2 diabetes are also on the rise. This field incorporates genomics, proteomics, and metabolomics and the effect of nutrients on them. Since it is now established that nutrition does have an effect on genes, research now focuses on how protein synthesis is affected. DNA microarray techniques, PCR, and electrophoresis are some of the tools used for research. SNPs may be one of the reasons why different individuals have varied reactions to the same kind of diet. For example, women who consumed a diet deficient in choline, and had MTHFD1-G1958A polymorphism had a higher chance of birthing a child with neural tube defects. The specific polymorphisms observed in certain populations make them predisposed to obesity. So it is extremely important to analyse the genome sequencing and what kind of diet triggers the expression of the particular gene **(Neeha et al., 2013).**

HUMAN HEALTH RELATED PROBLEM ASSOCIATED WITH AGEING

As already mentioned, people belonging to the older age groups have been classified into 3 classes.
With normal aging sensory deficits are common which include presbycusis or decreased hearing, presbyopia and visual acuity, and dizziness caused due to decline in vestibular function are seen. These conditions may or may not get worse with age. Loss in muscle mass and strength (sarcopenia) is caused due to impaired mitochondria and stem cell function in the muscle, changes in hormone levels, and chronic inflammation. This poses an impact on pharmacokinetics too. So, drugs are prescribed and administered accordingly. In hospitalised women, bacteria that do not cause infections colonise the urinary bladder due to which there is antimicrobial resistance.
Immunity also decreases to a large extent due to declining B-cell function, an altered T cell function, and improper functioning of the innate immunity. As antibody-production is lowered, vaccines are not as effective in people belonging to this age group. So, they are prone to various viral and bacterial infections that can consequently lead to complications.
Walking speed of old people also decreases. The walking measurement is used to predict various factors like likeliness to fall, or even mortality. In people belonging to the age-group of 85-89, men had an average walking measurement of 1m/s and women had an average of 0.8m/s. Whereas men above 90 years of age had an average of 0,9m/s and women had an average of 0.8m/s. Regular physical therapy and other physical interventions can improve speed and stability while walking. If a mobility disability is present, it is mostly due to factors like falls, depression, and social isolation. Most of the people above 85 who have disabilities, reportedly live alone.
Falls are also fairly common due to decreased balance and vestibular impairment. Almost 30-40% of people above 70 years of age have frequent falls. Injuries due to falls are high in adults above 60 years of age, and deaths due to falls are high in people above 85 years of age.
There is cognitive deterioration and the thinking capacity also reduces. Dementia and Parkinsonism are also common. They are neurodegenerative disorders, and their symptoms can worsen over time. As a result of the aforementioned conditions that are common with aging, adults of this age group generally slip into depression, and it is further worsened by factors like social isolation, retirement, and increased dependency **(Jaul Efraim and Barron Jeremy, 2017).**

EPIGENETIC MECHANISMS IN AGING

Aging is accompanied by certain physiological changes that act as hallmarks while analysing the process of ageing. They include – DNA methylation, modification of histone proteins, and ncRNA.

DNA Methylation

The "epigenetic clock" that depends on DNA methylation was observed to analyse its effect on ageing. Scientists have also discovered 9 hallmarks of ageing and have classified them into three categories – Primary, antagonistic, and integrative. Primary hallmarks include factors like loss of proteostasis, telomere attrition, and epigenetic alteration i.e., factors that cause cellular damage. Recent studies also suggest that epigenetic mechanisms dictate longevity. "Epigenetic drift" takes place as cell division progresses and there are stochastic changes in DNA methylation (DNAm) patterns **(Salameh Yasmeen et al., 2020).** CpG sites that undergo methylation are studied as they have a strong correlation with ageing, and it is relatively similar within the same species. Hence, these CpG sites are considered "Epigenetic clocks". Both epigenetic drift and epigenetic clock have detrimental effects on the tissues of organs, and accumulation of such damages can lead to organ failure. But some epigenetic changes are reversible and can help not only extend the lifespan but also delay the onset of health issues with age **(Ashapkin et al., 2019).** More specifically, CpGs methylated in EDARADD, TOM1L1, and NPTX2 genes were associated with ageing. Pathologically, mutations in EDARADD cause loss of hair, teeth, sweat glands, and also a slower rate of wound healing. There is upregulation of NPTX2 in Parkinson's disease, and expression of TOM1L1 is decreased in esophageal squamous cell carcinoma (Figure 1). Hypomethylation was observed in cultured human embryoblast lung fibroblasts under condition of senescence, both replicative and stress-induced **(Johnson wt al., 2012).** DNA methylation is affected by environmental factors. This reaction of DNA methylation is catalysed by the enzyme, DNA methyltransferase, and this covalent reaction is the addition of a methyl group on the cytosine ring in the 5' position. Similarly, DNA hydroxymethylation is the addition of a hydroxymethyl group in the 5' position of the cytosine ring, and this reaction takes place extensively in the brain especially in regions proximal to the synaptic genes. Studying these methylation reactions are important as they are a potential tool for predicting age and longevity. In fact, they are more accurate than prediction using the telomere length. DNAm biomarkers are also seen in age-related diseases like Alzheimer's, Cardiovascular Diseases, and Type-2 diabetes **(Salameh Yasmeen et al., 2020).**

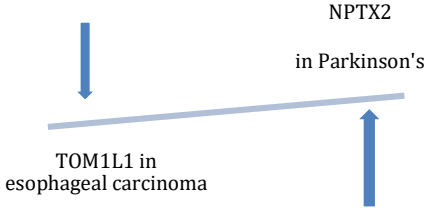

Figure 1 DNA methylation and age-related neurodegenerative conditions

HISTONE MODIFICATIONS

Histone modifications like "Histone methylation" are linked to lifespan regulation as they have an effect on transcription. Histone 3 Lysine 4 trimethylation and Histone 3 lysine 27 trimethylations are the two specific histone modifications. H3K4me3 methyltransferase along with the complex containing ASH-2 and WDR-5 affects life-span in worms. There was extension of lifespan when there was knock down of the methyltransferase subunit, whereas lifespan was shortened when the demethylase subunit RDR2 was knocked down. This indicated that methyltransferase positively regulates H3K4me3, and demethylase shows negative regulation of the same modification, and these have a direct effect on ageing.

On the contrary, H3K27me3 is a modification that is transcriptionally repressive. It is catalysed by the PRC 2 complex, and the modification is removed by UTX-1. When UTX-1 undergoes heterozygous mutation, there is an increase in levels of H3K27me2 and there is an insulin-dependent extension of life.

The effect of these modifications in flies have an effect opposite of that seen in worms. The overexpression of Lid (homolog of worm RBR 2) extends lifespan. This happens by the overall global increase in H3K4me3. But when there was a knockdown Trx model, no effect on life span was observed in male flies.

Mutations in H3K27me3 (PRC 2) subunits - E (z) and ESC showed a reduction in H3K27me3 levels and hence there was an extension of lifespan in male flies. However, a mutation in Trx suppressed the longevity because of increased levels of H3K27me3 levels of the E (z) mutant **(Sen Payel et al., 2016)**.

In yeast, an extension in life span was seen when there was ectopic expression of H3 and H4 histones. A reduction in histone biosynthesis was observed in senescent human fibroblasts which was caused as a result of histone depletion. When there was deletion of HHT1-HHF1 and a decrease in dosage of wild-type H3 and H4 by 85%, there was an increase in life span in yeast. Whereas, a 15% decrease in the dosage and deletion of the gene pair, HHT2-HHF2, showed a reduction in life span of yeast. This is because of blocking of TOR signaling as a result of moderate reduction in histone molecules H3 and H4 **(Yi Sun-Ju et al., 2020).**

In addition to methylation, histone ubiquitination also has an effect on ageing. This was studied in yeast that had components of deubiquitinase module or DUBm of the SAGA complex which included SCF73 (the ortholog of the human Ataxin-7 in yeast). The said protein acts as an adaptor molecule, linking SAGA complex with DUBm. It was found that mutant yeast strains that had a reduction in DUB function showed extension in life span as there was a lack of DUBm components. The main function of histone ubiquitination is the regulation of transcription and in DNA damage response. The basic structure of a chromatin is the nucleosome. The nucleosome consists of 147 base pairs of DNA wrapped around histone molecules. namely – H2A, H2B, H3, and H4 with two copies of each, hence it called the "histone octamer structure of DNA". H1 is the linker histone protein that helps anchor the DNA to the histone molecules. Heterochromatin is formed with the help non-histone proteins like heterochromatin protein 1 (HP1), along with H1 linker protein. "Heterochromatin loss model of ageing" is also a theory proposed to understand the contributing mechanisms of ageing. It states that there is a loss of nuclear architecture and hence expression of genes in a particular region of heterochromatin due to its degeneration along with ageing, and this leads to cellular senescence. Another result of heterochromatin degeneration is the loss of transcriptional silencing. Studies in eukaryotes (also studied in humans) suggest that accelerating this process causes a reduction in life span, and reversing it causes an extension in life span. When treated with histone deacetylase (HDAC) inhibitors, or when SIR2 genes (which code for HDAC) were deleted in yeast, there was shortening of life span. But overexpression of SIR2 results in an extension of life span **(Pal Sangita et al., 2016).**

NON-CODING RNAs

Neurodegenerative diseases and other age-linked conditions in organisms like yeast, mice, fly, and humans have all been linked to non-coding RNAs. Classes of ncRNAs include – miRNA, lncRNAs, rRNAs, tRNAs, etc. They are generally degraded quickly within the nucleus. But under certain conditions, processes that are toxic to the cells are triggered, in which one of them is the accumulation of genome-destabilizing R-loops. lncRNAs were found to be associated with ageing **(Szafranski Kirk et al., 2015).** More specifically, 9 lncRNAs were identified as "age-lncRNAs". When compared to protein coding genes, lncRNAs were more tissue specific. Using the Tau scores, it was also found that the down-regulated lncRNAs showed more tissue-specificity than up-regulated lncRNAs. Only one particular lncRNA was expressed in 5 tissues. They are also co-expressed with protein coding genes and GO and KEGG enriched terms. Up-regulated lncRNA and down-regulated lncRNA are expressed in different tissues and have an effect on the immune systems' function. One of the hallmarks of ageing is the dysregulation of immune system genes which are co-expressed with lncRNA. Tissues which were in contact with the external environment i.e., colon, lung, and esophagus consisted of down-regulated age-lncRNA with respect to immune system function **(Marttila Saara et al., 2020).**

INTRODUCTION TO *Drosophila* AND ITS HOMOLOGY TO HUMANS

Drosophila is an ideal model organism as it shares 60% homology with the human genome. Over 75% of the diseases seen in humans can be induced in the flies due to the homology. This makes studies quicker and it is ethically permissible.

Advantages of using *Drosophila* over rats:

a) They are easy and cost effective to maintain in large numbers.
b) They give rise to many progenies in a span of 2 weeks.
c) Their genome sequencing is easier.
d) Majority of the genes they possess are homologous with that of humans. **(Mirzoyan et al., 2019)**

Drosophila (Figure 2) have been used extensively for the study of drug delivery systems too. The strain the *Drosophila* possesses is important while replicating the human diseases. So, a fly with the yeast transcription factor, GAL4 (also present in yeast) expression driven by promoter regions only in specific tissues, and a fly with the response element of GAL4 (which is UAS) which is upstream of the transgenic element that is desired for the trial, were mated. In the transgenic progeny thus obtained, the expression of the transgene is defined by the promoter element of GAL4 **(Pandey et al., 2011)**

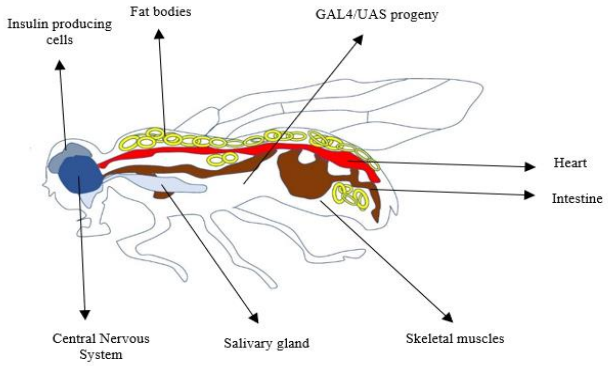

Figure 2 Parts of a *Drosophila*

ANTI-AGEING STUDIES IN *DROSOPHILA*

Gene Expression

Drosophila are the perfect models for Nutrigenomics due to the homologies of several human diseases present. As their life-cycles are short, anti-ageing studies are easier to observe. There are several pathways in *Drosophila* that are involved in nutrient signaling which also have an effect on the lifespan.

The characteristic of glucose homeostasis is partially conserved in *Drosophila*. 8 signaling insulin-like polypeptides (8 ilps), and PI3K are coded for by the *Drosophila* genome. When a signal is received, the transcription factor FOXO is phosphorylated and inactivated. They are equivalent to the function of both IGF-1 and relaxins in the mammalian insulin system. Knocking down of ilp 2 is sufficient to extend life-span. This effect is further enhanced by the knockdown of ilps 3 and 5. The aforementioned mutations make the flies more resistant to external and internal agents of stress like heat, ROS, lipophilic toxins.

The overexpression of FOXO gene (specifically in the gut and fat tissues) can extend lifespan. After deep-diving into the pathway responsible, 5 transcription factors were found to mediate the life-span extension. One of them is AOP, which is an ETS – family transcription repressor. Activation of AOP can be through inhibition of RAS/ERK pathway or by overexpression in the gut and fat tissues, and it plays in a role in extension of lifespan. While studying slow ageing in *Drosophila*, Obp996 gene was found to be upregulated and has now become a gene of interest to better understand the ageing mechanisms **(Piper et al., 2020).** The study suggests that regulation of the gene expression is preserved in aging *Drosophila*. The transcriptional activity of the flies was measured by assessing the expression of β-galactosidase, which is a reporter protein. This was done using enhancer-trap and reporter-gene techniques. When its expression was measured in the en (engrailed) gene of the fly antenna over the days, there was no change in the levels of expression. Similar results were seen in the Rh1-opsin gene. The enhancer-trap marked gene showed negligible change in the overall gene expression. Hence, it was concluded that there was co consistent change in the variation in gene expression **(Rogina Blanka et al., 1998).**

MITOCHONDRIAL DYSFUNCTION

Recent studies have shown that mtDNA mutations affect respiratory chain function. Phenotypes associated with ageing, like greying of hair, osteoporosis, are due to increased somatic mtDNA mutations. The basic principle is that the cells

that are deficient in respiratory-chain functions are more prone to apoptosis and hence there is cellular degeneration which presents itself phenotypically **(Trifunovic et al., 2008).** The GPCR BOSS (Bride of sevenless) is a ligand for sevenless tyrosine kinase. Boss mutant flies showed an increase in ROS, and also showed diminished locomotor performance and gut lipase function. Levels of ALEs and AGEs are elevated due to increased oxidative stress in these mutated flies. Regulation of ROS takes place by the expression of SOD2 present in the mitochondria, this expression was decreased in boss mutant flies. Oxidative stress contributes to various factors caused due to ageing like obesity and diabetes. A major type of ALE is 4-HNE, and its levels were elevated due to lipid peroxidation. One of the biomarkers of ageing in *Drosophila* is AGE, and its levels were also elevated.

SOD2 plays a major role in relieving the cell of oxidative stress. In the study, SOD overexpression was induced in boss mutation flies by crossing Act-Gal4 and UAS SOD2 flies. Then, oxidative stress levels were tested in the flies based on 4-HNE levels. It was found that SOD2 overexpression resulted in a decrease in 4-HNE levels. So, the study concluded that SOD2 plays a pivotal role in sensitivity of the boss mutated flies to oxidative stress **(Kohyama-Koganeya et al., 2013).**

When examining the structure of mitochondria in aged *Drosophila*, the cristae were found to be rearranged to form a "swirl". This was observed in young flies too when they were exposed to conditions of oxidative stress. The swirl also seemed to show deficiency in the said respiratory cytochrome c oxidase (COX). This factor is responsible for the increased vulnerability to apoptosis, especially in the flight muscles. There was a significant decline in complex IV COX activity with age, and when this was pharmacologically induced in young flies, there was an increase in ROS. So the oxidative stress is a cyclic event as impairment of mitochondrial function is ROS-induced, and this impairment further increases the ROS levels **(Cho Jaehyoung, Hur, 2010).** Cytochrome c oxidase (CcO) levels are also decreased in the ETC with age. CcO decrease will result in an overall decrease of ETC activity due to increased superoxide anions or hydrogen peroxide production in the mitochondria **(Peng Cheng et al., 2014).**

TELOMERASE ACTIVITY

The telomere present in *Drosophila* has similar functions, despite having a different mechanism. In contrast to human telomeres, *Drosophila* telomeres have retrotransposons which are associated with maintaining the length of the telomere.

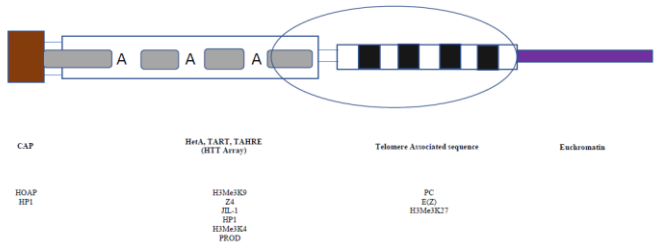

Figure 3 Structure of Drosophila telomere

The *Drosophila* telomere is divided into three regions (Figure 3) namely, cap, terminal DNA sequence that is present outside the cap, and the telomerase associated sequence (TAS). The H3MeK27 gene discussed in the previous topics is present in the TAS region. Delving deeper, the chromosome length is maintained by three telomere-specific non-long terminal repeat (LTR) retrotransposons which are – HeT-A, TART, and TAHRE. In human telomeres the length is maintained by short-repetitive sequences **(Mason et al., 2018).** Tel (Telomere elongation) is a dominant gene factor that causes lengthening of the HTT region by several-folds. But the study does not solidify that telomerase activity has an effecting on the longevity, but it does enhance fecundity **(Walter et al, 2007).**

LONGEVITY

a) Effect of high fat diet on life span

Drosophila that were subjected to a high-fat diet (HFD) showed increased mortality i.e., their life span was decreased (Figure 4). The nutrient medium consisted of 10% or 30% coconut oil. Just like factors like lethargy sets in humans whose diets predominantly consist of fats, flies also showed similar behavior. Their climbing activity, response to odor, sleep, and heart function decreased. HFD also had an effect on phototaxis memory, insulin signaling, and hence glucose homeostasis. Also, there is decreased transcriptional activity in genes that are associated with metabolism, memory, motor function, and cell signaling. Gustatory receptors present can sense free fatty acids (FFA), this sensing is affected when the flies are exposed to HFD for a longer-period of time. After one week of this diet, there was a decrease in body mass and an increase in triacylglyceride (TAG) levels. *Drosophila* insulin like peptides (DILPs) regulate lipogenesis and lipid storage after feeding. Akh and dilp levels were assessed and it was observed that Akh levels were elevated but dilp was not affected **(Liao et al., 2020).**

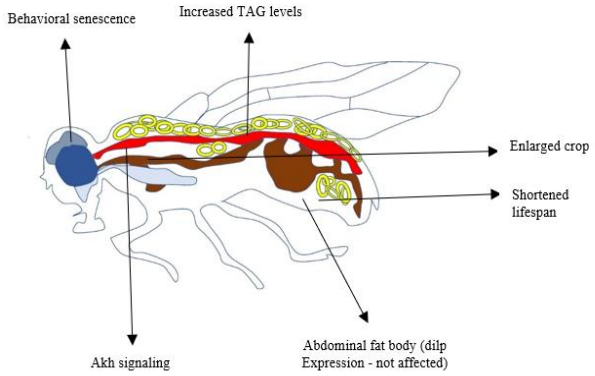

Figure 4 Effect of HFD on Drosophila

b) Effect of glucose on life span

When subjected to a glucose – enriched diet (GEF), lifespan of the flies was extended independent of any effect on the insulin pathway, by improving the integrity of the intestinal barrier of the gut. The ilps were quantified to further understand the mechanism. When ilp-2,3, and 5 mutants with GEF were studied, they were found to outlive wild-type flies. In the non-mutant flies, ilp-2, 5 levels were decreased slightly. Despite this, the insulin activity wasn't affected in this diet **(Galenza et al., 2020).**

c) Effect of amino acids on life span

The actual mechanism of the effect of amino acid on life span is still under study. But the TOR pathway is sensitive to amino acids. There are studies that suggest Methionine restriction results in extended life span in *Drosophila*. Met restriction has effects similar to that seen in dietary restriction (DR). Tsc2 or InRDN overexpression was seen to inhibit lifespan extension through Met restriction, but because of TOR signaling extension in lifespan is seen. Met restriction also reduced translation and reproduction which has a role in this extension **(Lee et al., 2014).**

NUTRITION FACTORS

a) Macronutrients

Ageing is essentially a process in which there is an accumulation of biomolecular wastes over a period of time, and the hallmarks of ageing like telomere attrition, mitochondrial dysfunction, epigenetic alterations, deregulation of nutrition signaling start manifesting. The nutrient signaling is primarily regulated by two pathways – IIS pathway and TOR pathway.

As discussed in the previous section, IIS pathway is characterised by the presence of 8 ilps, and bind to InR. The Insulin-producing Cells (IPCs) are presence in the *Drosophila*'s brain. When the sugar levels are low, APMKα is activated and it signals the release of adipokinetic hormone (Akh), which regulates glycemia by binding to AkhR and converting glucose and lipids to free energy. Ilps secretion is regulated mainly by fat body present in *Drosophila*, and indirectly affects longevity. If the IIS activity is reduced, fertility is also affected. IIS pathway has an effect on longevity through metabolic homeostasis. The cncC/Nrf2 pathway, which is activated by IIS, is sensitive to the nutritional status and helps in detoxification which results in the delayed onset of age-related conditions.

Amino acids are sensed by TOR signaling pathway. They play an important role in anabolic processes like protein synthesis, but it also required to balance the catabolic processes. When TOR was inhibited through nutrition longevity is promoted and IIS pathway is also suppressed. When Tor present in the fat body senses (Figure 5) the nutrient, a humoral signal modulates IIS and growth is observed in the peripheral tissues **(Evangelakou et al., 2019).**

mTORC1 and mTORC2 are the two mTOR complexes. They share mLST8 and DEPTOR, but mTORC1 exclusively has PRAs40 and RAPTOR, whereas RICTOR, mSIN1, and Protor-1/2 are found in mTORC2. Amino acids, growth factors, and oxygen are the factors which evoke a response from mTORC1, and mTOR-dependent anabolic processes like protein synthesis is initiated. PI3K/PDK/AKT regulated signal pathways (for example, IGF and insulin) inhibit TSC1 and TSC2, thus activating mTORC1. One of its effectors is S6K. On the contrary, mTORC2 is responsible for regulation of cytoskeletal organization and in activation of AGC-family kinases, which includes AKT and SGK 1, which also

negatively regulates the FOXO1/3A. In the presence of amino acids mTORC1 is activated by RAG-GTPase, which results in the formation of heterodimers – A/B, C/D. These active heterodimers are bound to GTP (A/B) and GDP (B/C) recruit mTORC1, which is then activated by RHEB.
So, signals of both IIS and TOR work cohesively in the presence of nutrition **(Papadopoli et al., 2019).**

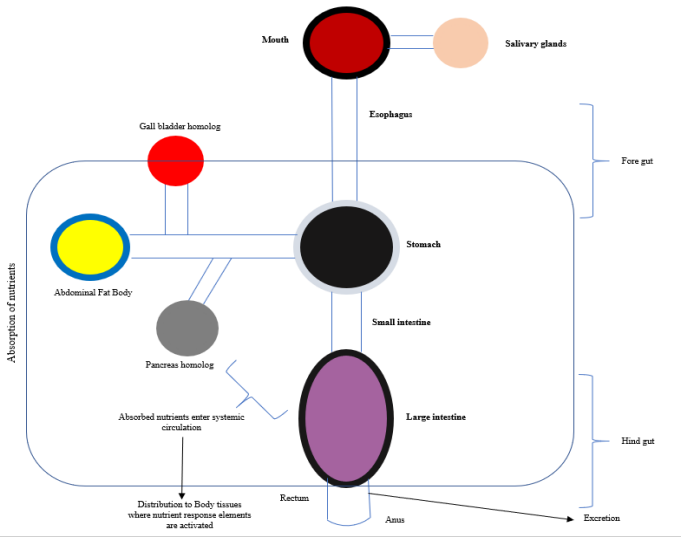

Figure 5 Digestive system of *Drosophila*

b) Dietary restriction

Many studies have shown that a reduction in the overall dietary intake can extend lifespan in *Drosophila*. This reduction is called dietary restriction.
It can also be through dilution of the nutrient medium. Progressive dilution has shown maximum extension in lifespan, but if the dilution exceeds the optimal concentration there will be a reduction in life span due to starvation. The mean and maximum life span of the test population is increased, but the increase is more in females than in males. Again, IGF-like signaling and TOR pathways play a role in extension due to DR, and this effect is further catapulted by the presence of dSir2 and Rpd3. But there was no significant effect on oxidative stress and mitochondrial dysfunction, the exact mechanism is still under study **(Partridge et al., 2005).**
To make sure that IIS pathway is involved in life span extension due to DR, chico mutant and FOXO mutant flies were subjected to the same DR. There was no significant change in the lifespan in chico mutants, but there was an extension in foxo mutants which indicates the involvement of IIS pathway.

BIOACTIVE COMPONENTS AND THEIR MECHANISMS

Bioactive components (Eg – antioxidants) have been proven to extend life span by reducing oxidative stress on the cells. A few of the components also directly act on genes and regulate them (Table 1).

Plant extracts

Berries and fruits like apple **polyphenols** extend mean life span of *Drosophila* by 10%. This because of upregulation of superoxide dismutase (SOD) and catalase (CAT), and the downregulation of mth and Rpn11.
Rosemary had a dose-dependent effect on the lifespan extension. At 3mg/mL, the maximum longevity was extended by 12%. It also prevented lipid peroxidation thus decreasing the oxidative stress and also decreased malondialdehyde (MAD). It also increased CAT and SOD activity.
When flies were fed with 1mg/mL of **ginger extract**, CAT and SOD where upregulated. But when 2mg/mL of extract was fed, mth was downregulated. So, the 1mg/mL and 2mg/mL extended the maximum lifespan by 11.97% and 4.66% respectively.

Aronia extract is a good antioxidant. 2.5 mg/mL of this extract was fed for 40 days and it improved the locomotor activity. The maximum life span was increased by 18%. Due to the accumulation of MDA, ROS production was also decreased. The associated enzymes and genes with this extension in longevity and improved locomotion are the antioxidant enzymes CAT, SOD, and GPX (Glutathione peroxidase), and the stress resistant genes Hsp68, l(2)efl (Lethal 2 extension for life), and Jafrac1 **(Evangelakou et al., 2019).**

Plant derived compounds

Resveratrol, when fed to *Drosophila*, did not seem to show any significant changes in the extension of maximum life span. The lifespan extension was dependent on Sir2, which is a deacetylase. There was no response towards hydrogen peroxide **(Bass et al.m 2007).**

Ursolic acid is a triterpenoid and has anti-inflammatory, antibacterial, and anti-obesity properties. There was an extension of life span by the increase in levels of srl (Spargel). This increased the climbing activity of *Drosophila*. The upregulation of srl/PGC1 did not affect the fecundity and gut integrity of *Drosophila* **(Staats et al., 2019).**

Epigallocatechin gallate extended life span in male flies by 50%. There was upregulation of SOD and CAT. The additional factor that helped in this extension was that EGCG also prevented accumulation of iron. α-amylase and α-glucosidase activities were inhibited, which decreased glucose concentration. Also, ilp5, phosphoenolpyruvate carboxylase (Pepck), and upd2 expressions were suppressed. These genes are responsible for regulating energy homeostasis and glucose metabolism hence they contribute in improving fitness of the flies **(Evangelakou et al., 2019).**

OTHER MODEL ORGANISM IN NUTRIGENOMICS RESEARCH

Other than *Drosophila*, *Caenorhabditis elegans* and mice have been used as model organisms for research. *C. elegans* have been used to study the general effect of nutrition. Since the genetic homology between humans and *C. elegans* are not much, the results are not applicable to human studies. Since the study of nutrition is also based on phenotypic manifestations, *C. elegans* can act as an appropriate model to identify genes that are responsible for the particular change. The required source for RNAi is also available. The desired mutants are generated by CRISPR/Cas9 method. While considering the signaling pathways in *C. elegans*, the JNK, AMPK, TOR, and autophagy are conserved in humans up to the molecular level. But this is where the similarities between humans and worms end. So, in this context the results cannot be applied in human studies. **(Gottschling et al., 2019).**
Rats and mice are more similar to humans in terms of genetic variations. Since humans and rats tend to prefer the same kinds of food, they are not as nutrition specific as *C. elegans*. Their RNA profiling can be done easily, and their genes can be manipulated to analyse the human diet. But their life cycle takes longer, and hence the time of research is much more when compared to *Drosophila*. The number of progenies is also very high in rats and mice **(Reed, 2008).**

NUTRIGENOMICS STUDIES IN HUMAN INTERVENTION STUDIES

When studying nutrigenomics in humans, fields of proteomics, transcriptomics, and metabolomics and their profiling studies are included. Due to genetic polymorphisms present, there is heterogeneity that has to be considered when it comes to the relevant genes that are involved in the biological processes and pathways **(Wittwer et al., 2011).** The fields of nutrigenomics and nutrigenetics are important fields of research as they work towards better understanding the effect of nutrition on humans at the genetic level. To explain this, there should be a complete understanding of the biochemistry, genetics, and molecular make-up of humans. High-efficiency studies are important for profiling individuals. But the cellular and molecular components of humans are extremely complex and a lot of the mechanisms are still insufficiently explained **(Panczyk, 2013).**

Table 1 Bioactive compounds and its mechanisms

FOOD	MECHANISM	LONGEVITY
Berry and apple	Upregulation – SOD, CAT Downregulation – mth, Rpn11	Extended by 1%
Rosemary extract	Increased - SOD, CAT Prevention of lipid peroxidation Decreased MAD	Extended by 12%
Ginger extract	Upregulation – SOD, CAT (1mg/mL) Downregulation – mth (3mg/mL)	Extended by 11.97% Extended by4.66%
Aronia extract	Improved locomotor activity Decreased ROS production, accumulation of MDA Upregulation – SOD,CAT,GPX, Hsp68, l(2)efl, Jafrac2	Extended by 18%
Resveratrol	Sir 2	No significant changes
Ursolic acid	Increased – Srl Increased climbing activity	Extension of lifespan
EGCG	Prevents accumulation of iron Inhibition of α-amylase and α-glucosidase Suppression – ilp5, Pepck, upd2	Extension of lifespan

CURRENT STUDIES AND FUTURE APPROACH

Nutrigenomics is the next big field of interest in the food industry. At present, scientists are focused on the epigenetic biomarkers as they have proven to play a critical role in studying the effect of nutrition on the genes. This will also enable the development of "personalised diets" since the reaction to nutrition varies even among a population. Studying the effect of bioactive ingredients on the biomarkers has also become a top priority for scientists. The genome-wide study of nutrition and the metabolic stress, insulin resistance, and the genesis of metabolic syndromes by metabolic stresses. Diets should purely be for health and they should be complementary to the pharmacological therapies. For this to be possible, new genomic-biomarkers have to be identified and their specificity to the diseases that they cause should be studied more deeply **(Neeha et al., 2013).**

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