Effect of Dietary Restriction on Muscle Performance in *Drosophila*

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Abstract

Dietary restriction (DR) can extend the lifespan in various organisms and is thought to present potential benefits in human. However, it is still a controversial issue now among scientists due to lack of strong supportive evidence in human. Aging is an important issue in every developed country, and the aging process causes neurodegeneration and sarcopenia, (the degeneration of muscle mass, strength and function). The aim was to understand sarcopenia in the model organism, *Drosophila melanogaster*, by monitoring the decline in output of a single twitch muscle, the jump muscle, with age. Jumping assays were performed using an ergometer, while the flies were fed with different yeast concentrations throughout their adult life. We did not observe failure of neuronal conduction as the flies aged; rather the muscle performance declined gradually with age. Flies fed with reduced yeast concentration showed significant extension of lifespan in both *Canton-S* or *Canton-S*/Wee-*P* flies but no difference in jumping performance. However, flies raised in high yeast concentration both from larval stage or adult stage had shorter lifespan without any reduced jumping performance.

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Chapter 1 Introduction

Aging is correlated with the loss of neuromuscular function and structural integrity that often results in a substantial decline in muscle strength and mobility (Doherty, 2003). The loss of skeletal muscle mass, strength and function associated with aging is commonly known as sarcopenia. As the proportion of the population over 65 increases, the medical, social and financial consequences of muscle failure will increase, so any measures that can be taken, e.g. changes in diet, to improve muscle function are to be welcomed.

1.1 Theories of causing Aging

Aging involves an array of complex mechanisms at different levels. The first point taken into account will be the theory of molecular mechanisms, based on age-related cellular deterioration.

1.1.1 Theories with Molecular Mechanism of Aging

1.1.1.1 Somatic Mutation Theory

Previous studies implicate the capacity for DNA repair as a key factor for aging at the cellular and molecular level. The relationship between lifespan and DNA repair is exemplified by some enzymes, such as ADP-ribose and PARP-1, which play an important role in the immediate cellular response to stress-induced DNA damage (Promislow, 1994; Burkle, 2001).

1.1.1.2 Telomere Loss Theory

In some human somatic tissues, the integrity of chromosomes is maintained through the presence of telomeres, as well as recombination processes. The length of telomere is a clear age marker becoming gradually shorter when cells divide (Kim *et al.*, 2002). The loss of telomeric DNA is usually called the “end replication” problem

because the newly synthesized DNA is shorter at the 5' end compared to the original DNA template, due to shorter telomere at the end of the strand (Kirkwood, 2005); however, this situation occurs only in eukaryotes. Some *in vitro* studies have shown that the rate of losing telomeres is influenced by stress especially oxidative stress (von Zglinicki *et al.*, 1995, 2000). Accelerating or slowing different levels of stress in cells has a great effect on shortening the length of telomeres.

1.1.1.3 Mitochondrial Theory

Mitochondrial DNA (mtDNA) mutations have an important relationship with molecular stress and aging (Wallace, 1999; Kirkwood, 2005). The accumulation of somatic mtDNA mutations will ultimately reduce the energy output below the needed level and results in loss of memory, hearing, vision and stamina as aging occur. The activity of a main mitochondrial respiratory-chain enzyme called cytochrome *c* oxidase (COX) has a close relationship with mitochondrial DNA deletion which greatly accumulates in human muscle (Müller-Höcker, 1989; Brierley *et al.*, 1998), brain (Cottrell *et al.*, 2000a, 2000b), and gut (Taylor *et al.*, 2003), cells where COX is deficient. Cells with normal COX activity have less mitochondrial DNA deletions than cells with low COX activity, where the latter one is prone to cause age-related diseases (Bender *et al.*, 2006).

1.1.1.4 Altered Proteins Theory

Protein turnover, whereby damaged or redundant proteins are removed, is essential to maintain cellular homeostasis and regulate the multiple cell functions. The rate of protein turnover is related to the rate of protein synthesis and degradation. Some experimental evidence showed that an accumulation of altered proteins and protein turnover led to a variety of age-related neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease and cataract. Protein turnover has the aid of

chaperones, to protect against the aggregation of proteins, by clearing damaged proteins, (Söti and Csermely, 2007, p.511), restore denatured proteins and target to proteasomes (Söti and Csermely, 2000, 2003, 2007; Young *et al.*, 2004).

1.1.2 Network Theories of Aging

Multiple aging mechanisms are now widely acknowledged. Whilst studies have shown molecular or cellular damage during aging, none of them sufficiently connect to age-related weakness, disability or disease in any of the theories. The development "network theories" of aging has implicated various mechanisms working together, with different processes interacting or acting synergistically (Kirkwood et al., 2003). For example, the accumulation of mitochondrial DNA mutations might cause an increasing production of reactive oxygen species (ROS), decreasing the energy output occurring over time. Understanding these connections will help develop understanding of age-related cellular deterioration.

The other approach of network theory is that although many mechanisms within the network are conserved in all cell types and species, many have differences relevant to which mechanisms are more important to that cell type. All cells share a basic common repair mechanism for damage affecting primary macromolecules such as DNA and protein, specifically when the damage appears from normal sources such as endogenous oxidative stress caused by ROS. However, although the network of mechanisms may share similar common components across all cell types, consideration of differences may apply to different species (Kirkwood, 2005).

1.2 Muscular Aging

Sarcopenia is a gradual and long term irreversible loss of skeletal muscle mass, strength and muscle function, which occurs during aging in many different living

organisms (Fang *et al.*, 2011). During the aging process, age-related loss of muscle strength occurs with either the loss of muscle mass, or the decrease in muscle-specific force. The oxidative damage and repair system will slowly fail during aging. The loss of skeletal muscle capacity and loss of locomotory abilities are the typical influences during aging and we will focus on muscle and neuronal degeneration. The muscle output will be measured as a monitor of this degenerative process. The first question we would like to address will be the causes of sarcopenia, based on age-related loss of skeletal muscle.

1.2.1 Skeletal strength and muscle mass loss in aging

Loss of skeletal strength is a common sign resulting from aging and also directly impacts and relates with loss of skeletal muscle mass. The evidence shows that male subjects were physically fitter than females at all ages; the loss of muscle strength was similar for both male and female subjects and the decline of these were similar for both evoked and voluntary contraction determined by examining the ankle plantar flexor and dorsiflexor muscles of adolescent, middle-aged, and aged male and female subjects (Vandervoort and McComas, 1986). Most elderly male and female subjects were able to ultimately activate less motor neuron pool for producing the maximum energy. Overall, the losses of proximal and distal muscles in the upper and lower extremities were being lost at similar levels, and both male and female subjects had similar muscle losses.

Similarly, male subjects had obviously higher skeletal muscle mass than females with more loss of skeletal muscle mass during the aging process, determined by examining several different parts of muscles with 148 female subjects and 136 male subjects aged between 20 and 90 years old (Gallagher *et al.*, 1997).

1.2.2 Muscle quality changes in aging

Muscle quality (MQ) is related to the strength per unit cross-sectional area (CSA) or strength per unit of muscle mass and is more related with muscle function than only strength (Roubenoff and Hughes, 2000). Some MQ experiments with different genders during aging have been reported. For example, arm MQ is greater than leg MQ looking at groups of all ages (Lynch *et al.*, 1999). These age-related losses of muscle quality may relate to some factors, such as changes in neural drive, altered muscle pennation and increases in connective tissue. Moreover, the expression of myosin heavy chain (MHC) in muscle fibres from adolescent male subjects was healthier (decline lower) than elderly males, and type I and IIA fibres from elderly males were healthier than elderly females (Frontera *et al.*, 2000). These findings showed clearly that there were differences in MQ between older men and women (Doherty, 2003). Also, there is another experiment showing the changes in expression of MHC in the slow twitch soleus (Sol) muscle shifts to the opposed direction compared to glycolytic or fast twitch muscles, such as gastrocnemius (Gas) muscle, in rats during aging (Edstrom and Ulfhake, 2005; Snow *et al.*, 2005). By comparing the phenotypic changes in slow twitch Sol muscle and fast twitch Gas muscle at advanced stages of sarcopenia, the evidence reveals that there are large phenotypic shifts in both twitch muscles with aging (Carter *et al.*, 2010).

Drosophila skeletal muscle has similar energy generation and muscle contraction mechanisms compared with humans, so that it is a suitable model for muscle aging research (Miller *et al.*, 2008). Muscle degeneration can be divided into two main reasons: external parts include lack of physical performance and nutrition; while the internal parts include a decreased level of circulatory anabolic hormones, increased apoptosis caused by raised incidence, accumulation of mitochondrial DNA mutations,

reduced oxidative capacity, and muscle regenerative properties declined (Augustin and Partridge, 2009).

1.3 Neurodegeneration

Muscle loss is not the only factor responsible for physical damage. Neurodegeneration could be the other cause of physical deterioration during aging process. During the aging process, brain function is significantly reduced. Several reasons account for the loss of neurons, which leads to neurodegeneration. Other reasons, which can relate to aging brains, includes oxidative stress, reduced innervations and genetic defects (Sang and Jackson, 2005).

There is much debate as to whether muscle atrophy results from when muscles start to degenerate as innervations degenerate. It was reported in mice that after loss of innervation, the muscle will start to degenerate (Muller *et al.*, 2007). However, it is still not clear in *Drosophila*. There are few experimental studies focusing on neural function and muscle activity using *Drosophila* as a model. Superoxide dismutase (SOD) enzymes work together with catalase to de-toxify ROS in cells, which have been reported to extend the lifespan in flies (Parkes *et al.*, 1998) and in mice. The skeletal muscles were identified as the primary target for $SOD1^{G93A}$ mutant protein toxicity due to muscle atrophy (Dobrowolny *et al.*, 2008; Godenschwege *et al.*, 2009) but the detail for muscle degeneration before neurodegeneration or vice versa are still unknown in flies.

1.4 Lifespan Studies

Over the past hundred years, the general lifespan of human beings has increased extensively in the world due to the medical and environmental improvement. Many types of interferences, genetic manipulations and caloric restriction (CR), have been

reported to increase the maximal lifespan in many different species. However, the results from these experiments are to be determined whether or not it will carry out the ultimate benefit to prolong the human lifespan. Several hypotheses (**Table 1.**) have been posed to illustrate the biological aging process (Vendelbo and Nair, 2011). In *Drosophila melanogaster*, the response of lifespan in dietary restriction is regulated by nutrient-sensing pathway, which include the Target of Rapamycin (TOR) (**Figure 1.**) (Hansen *et al*., 2007; Kapahi *et al*., 2005; Kapahi *et al.*, 2004), adenosine monophosphate-activated protein (AMP) kinase (Greer *et al*., 2007), sirtuins (Rogina and Helfand, 2004; Li *et al.*, 2008) and Insulin/Insulin-like growth factor (IGF-1) signalling (Honjoh *et al.*, 2009; Arum *et al.*, 2009).

Table 1 Hypotheses explain biological processes regarding senescence.

Key hypotheses in aging

- Mitochondrial function and ROS formation
- Caloric restriction (CR)

ROS scavenging
tissue development
energy metabolism
} signal transduction
stress response
structural and contractile proteins ⇩

Lifespan can be extended by several conditions, such as heat, oxidative stress, low ambient temperature, chemosensory signals, thermosensory signals, signals from the reproductive system and reductions in the rates of respiration or translation. In these conditions mentioned above, the response of lifespan is under active control by specific regulatory proteins (Kenyon, 2010).

Some tissues (e.g. adipose tissue) also have a predominant role in prolonging the lifespan of *Drosophila melanogaster* (Libina *et al.*, 2003; Wang *et al.*, 2005; Wolkow *et al.*, 2000). For example, *FOXO* proteins are a subgroup of the forkhead family of transcription factors. The overexpression of *FOXO* in *Drosophila* fat bodies extends lifespan, which points out a main role of this tissue when regulating the lifespan (Giannakou *et al.*, 2004; Hwangbo *et al.*, 2004). In *Drosophila*, *FOXO* is a key component of the insulin signalling cascade and by binding directly to target gene promoter regions, it regulates the expression of various numbers of target genes such as those regulating metabolism, cell growth, cell proliferation, stress resistance, and differentiation (**Figure 2**) (Salih and Brunet, 2008). In adipose tissue, an increase in *Foxo* activity prolongs the lifespan (Fabio and Perrimon, 2010).

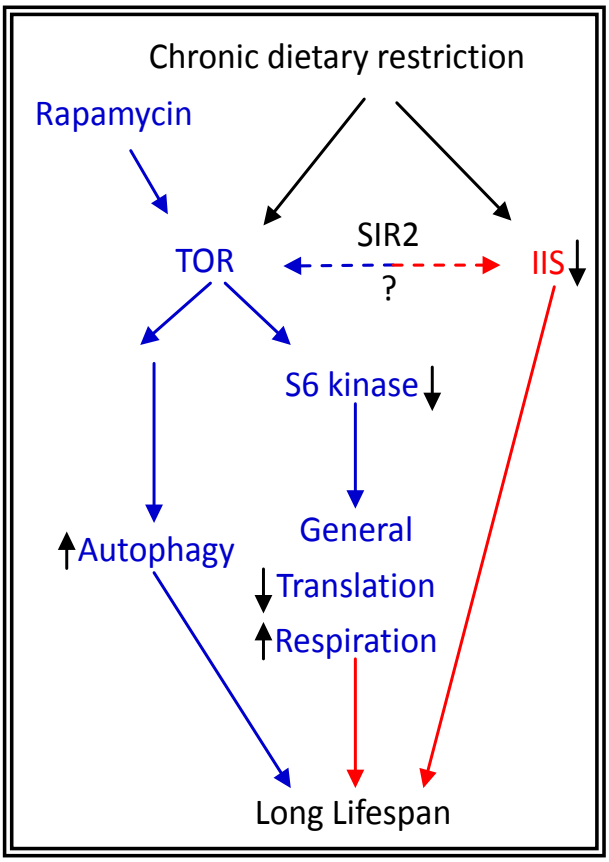


Figure 1

Pathways that extension of lifespan influenced by responding to chronic dietary restriction in *Drosophila melanogaster*. By downregulating TOR activity, chronic dietary restriction increases lifespan. (Modified after (Kenyon, 2010))

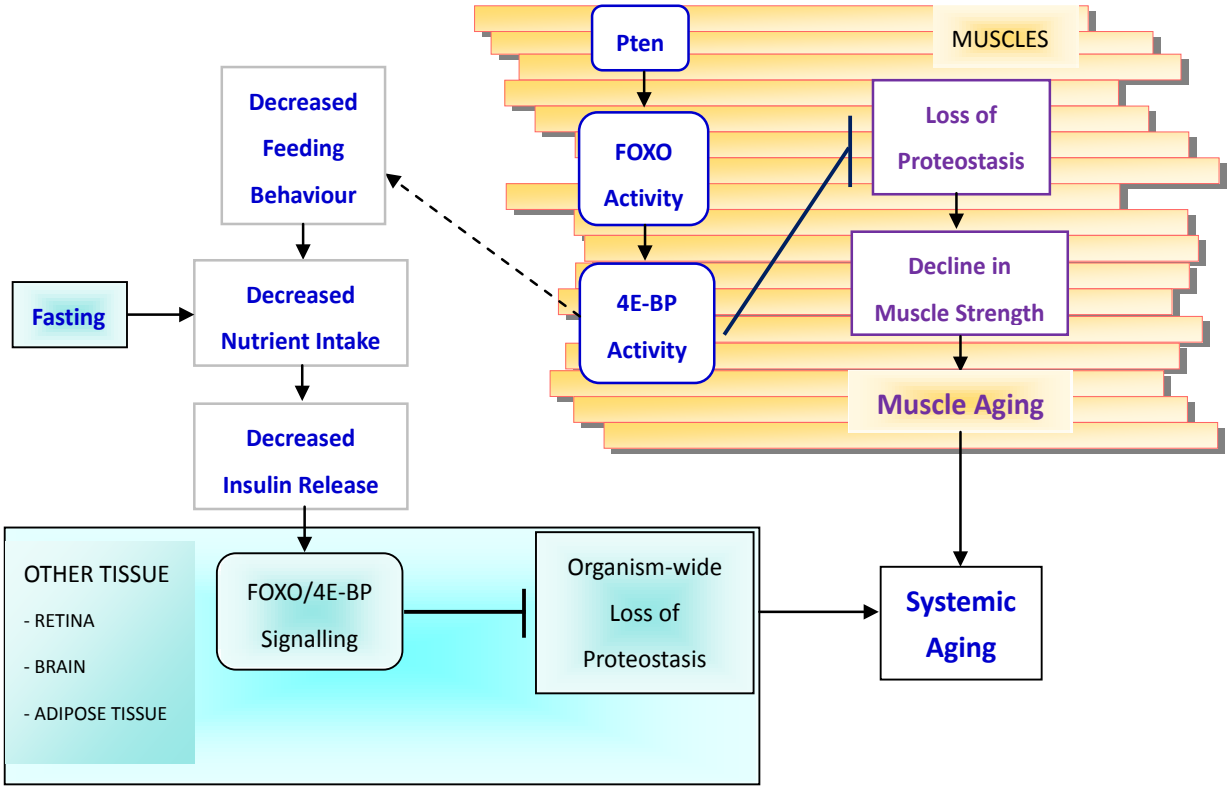


Figure 2
Regulation of FOXO/4E-BP signalling in muscles and other tissues. FOXO/4E-BP activity regulates muscle to maintain muscle function, and prolong the lifespan by autophagy/lysosome pathway (Modified after (Demontis and Perrimon, 2010))

1.5 Dietary Restriction

Without losing key nutrients and minerals, dietary restriction (DR) is described as a reduced consumption of particular nutrients or total nutrient absorption. In the general broad definition, DR may include caloric restriction (CR), but the same calories from different nutrients may lead to different results, which may correlate to feeding behaviour and physiology in living organisms (Katewa and Kapahi, 2009). In 1935, the first effect of DR on extension of lifespan was reported in rodents (McCay *et al*., 1935), whilst the reduction of nutrient ingestion has been shown to prolong lifespan in a variety of invertebrate species. DR has turned into a main study in many living organism models, including *Saccharomyces cerevisiae* (Jiang *et al*., 2000, 2002; Lin *et al*., 2000, 2004; Anderson *et al*., 2003; Kaeberlein *et al*., 2004; Sinclair, 2005; Guarente, 2005; Piper, 2006; Kaeberlein *et al.*, 2007; Dilova *et al.*, 2007; Longo, 2009), *Caenorhabditis elegans* (Klass, 1977; Johnson *et al*., 1990; Lakowski and Hekimi, 1998; Houthoofd *et al*., 2003; Walker *et al*., 2005; Schulz *et al.*, 2007) and *Drosophila melanogaster* (Partridge *et al*., 1987; Chippindale *et al*., 1993; Chapman and Partridge, 1996; Pletcher *et al.*, 2002; Mair *et al.*, 2003, 2004; Piper *et al*., 2005, Piper and Patridge, 2007). For these living organisms to survive DR conditions, resources may be diverted from reproduction to somatic maintenance. The effect of DR in *Drosophila* is between starvation resistance and lifespan. Flies in high yeast concentration laid many eggs but lived with short lifespan after food deprivation; however, flies in low yeast concentration laid fewer eggs but survived with longer lifespan (Chippindale *et al.*, 1993). Moreover, by varying all components of adult diet (yeast and carbohydrate), the intermediate food concentration resulted in the longest lifespan in female flies, but the level of egg production was increased similarly followed by the level of food concentration (Chapman and Partridge, 1996). It seems

the resource allocation of *Drosophila* shift is due to the somatic function when under DR (Good and Tatar, 2001).

Preliminary studies also suggest that DR may extend lifespan in other organisms (Roth *et al*., 1999; Lane *et al*., 2000) and have potential benefits in human beings (Fontana *et al*., 2004). In 2006, some biogerontology scientists argued a question: "Do you think that DR can increase longevity in all species, particularly in human beings?" (Bourg and Rattan, 2006, p.124) This question led several experts from different backgrounds to a wide discussion. It came out with three different conclusions. Firstly, it is too early to decide. There was an experiment that showed DR failed to extend lifespan in DBA/2 mice strain (Sohal *et al.*, 2009), and the result in flies has been unclear yet. It seems that DR is not the only condition that would influence the lifespan. There might be having a species-species problem, so that it is too early to mention the potential positive effects of DR in human beings. Secondly, some experts believed that DR could be of benefit for human beings. They claimed that although DR did not increase lifespan in monkeys which were older than 15 years at the early stage of study, these monkeys showed improvement when they were in DR, such as less diabetes, cancer, heart and brain diseases (Nicholas, 2009). Therefore, people would never know the result for the impracticability of DR experiments on human beings. Also, some people think that DR seems to have potential improvement of protecting against age-related diseases in human. Moreover, DR may also improve human being's health and increase their average lifespan, as it affects many species. For example, DR already improved the extension of lifespan on people who live in Okinawa, Japan. It is clear that having a large amount of fruits and vegetables could positively extend the lifespan in humans. However, it was mentioned that DR cannot function for human beings by Bart Braeckman groups, which they proposed that DR

does not have any effect on species with an aging sexual maturity, small offspring size and long lifespan. Also, in Okinawa's case, the daily energy provided was 2800 kCal, which was nearly 20% less than the average of developed countries, and that the average lifespan of women in Japan was 85 years old (Sataro Goto, 2006). Therefore, DR is not a convincible subject on increasing the lifespan in human beings from this example. Thus, they claimed that bigger-sized and long-lived primates are less susceptible to a temporary food shortage than smaller-sized and short-lived ones, since the former can at least depend on nutrients stored in the body for a short period during the shortage of food (Bourg and Rattan, 2006).

1.6 *Drosophila* as a Model

Drosophila has many advantages as a useful experimental model organism for investigating the prolongation of lifespan and the effect of DR during the aging process. Their short life cycle allows lifespan and aging experiments to be under taken in a short period. They are sexually dimorphic (males and females are different individuals), males and females are clearly distinguishable and virgin females are easily isolated, also helpful for lifespan assays and aging experiments. They are an excellent model for genetic and molecular manipulation, easy to care for and culture in the laboratory and they are obligate aerobes, which may cause similar aging-related damage during the aging process (Partridge *et al*., 2005). The developmental period for *Drosophila* varies with temperature. Adult flies live about 30 days at 29 °C, under ideal conditions. Though they can live longer at lower temperatures, changes in lifespan can still be observed at 29 °C during the aging experiments. (Loeb and Northrop, 1917; Ashburner and Thompson, 1978; Ashburner *et al*., 2005)

From many previous studies, *Drosophila* flies work well as a DR experimental model. Especially, the lifespan of flies is obviously maintained longer in the yeast diet

treatments than in the sugar diet treatments. The effect of calories from the metabolites specific to yeast can be categorized into carbohydrates, sterols, fatty acids, vitamins, minerals and amino acids. Amino acids can be seen as the most important components because there were some experimental results showing that decreasing the quantity of methionine can extend the lifespan either in rats or mice (Miller *et al.*, 2005; Zimmerman *et al.*, 2003). It perhaps leads to similar effects in flies (Min *et al.*, 2006).

1.7 Muscle Measurement

Muscle degeneration occurs throughout the aging process. The aim of my study is to understand the relationship between age and decline in jump performance by measuring the muscle output of *Drosophila* jump muscle at different time points. The measured muscle will be the *Drosophila* jump muscle, the tergal depressor of the trochanter (TDT), also named the tergo-trochanteral muscle (TTM) (**Figure 3.**). This muscle produces a single twitch for each stimulus and also the size of this muscle is bigger than other muscles, which is responsible for movement of the legs (Harvey *et al.*, 2008).

The jump pathway can be activated by an electrical stimulus to the head, causing an action potential in giant descending neurons (GDN), which follows generating an action potential in the TDT motorneuron and Peripherally Synapsing Interneuron (PSI) interneuron. The PSI next excites the motorneurons supplying the indirect flight muscles (IFM). The TDT contracts, the legs are extended and the wings move into position for flight, so that when the IFMs are activated, flight begins (**Figure 4A and B**) (Allen *et al.*, 2006).

Moreover, Wee-*P* 26 flies (Clyne *et al.*, 2003) can express green fluorescent protein (GFP), and the only thoracic muscle that expresses GFP in the Wee-*P* flies is

the TDT; other muscles (in the leg) also express GFP, so that it might be useful in monitoring the jump muscle size.

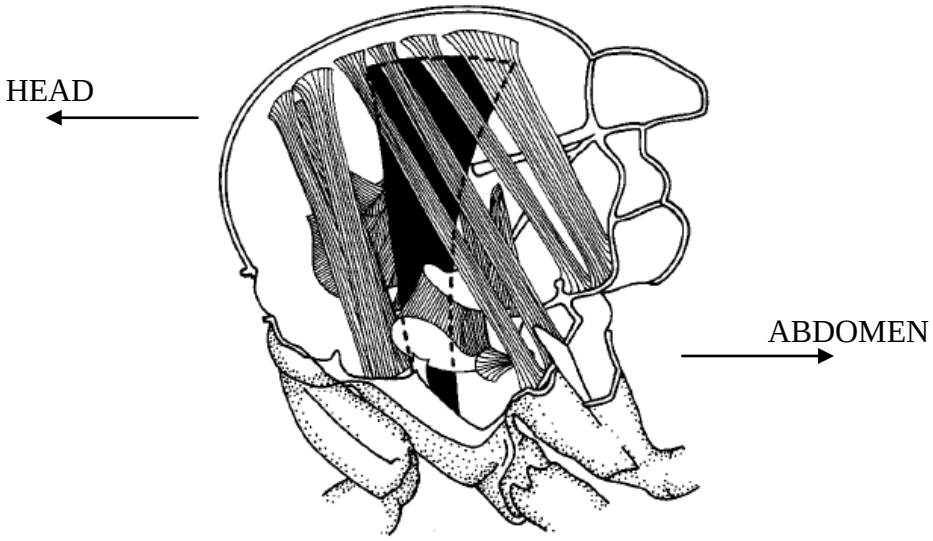
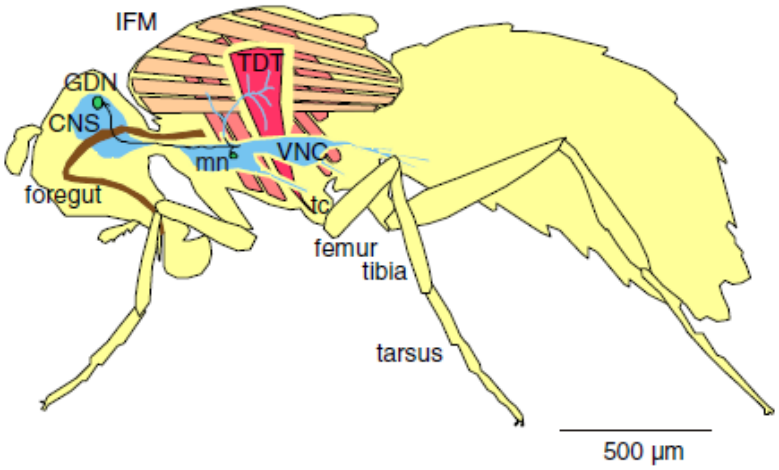


Figure 3
The tergal depressor of the trochanter muscle (TDT) (black field) in the thorax of *Drosophila melangaster*. (Modified after (Peckham *et al.*, 1990))

A



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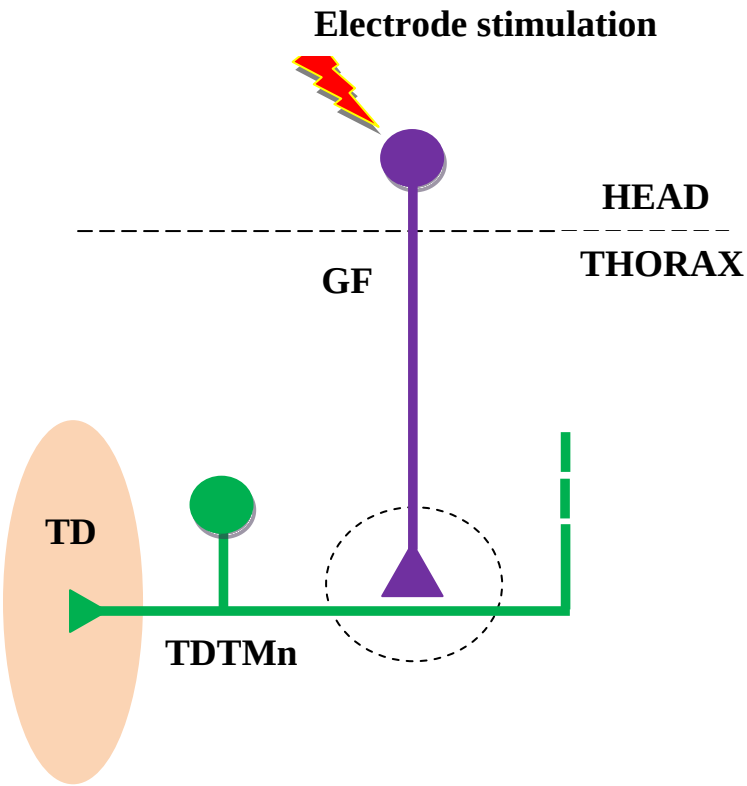


Figure 4

Pathway of jumping performance in *Drosophila melangaster*. **(A.)** The organisation of the jumping pathway. (Harvey *et al.*, 2008) **(B.)** Simple representation of the synaptic connection involved in the stimulation of the TDT during the experience. Stimulation of the GF will active the TDTMn. The GF-TDTMn synapse is represented by a dotted circle (purple and green). This will finally result in the contraction of the TDT. Only one side of the fly has been represented for clarity. (Modified after (Allen and Murphey, 2007)) (GDN: Giant Descending Neuron; VNC: Ventral Nerve Cord; TDT: Tergal Depressor of Trochanter; IFM: Indirect Flight Muscles; GF: Giant Fibre; TDTMn: Tergal Depressor of trochanter motoneuron)

Chapter 2 Aim of the Project

The aim of this project is to observe the effect of dietary nutrients in a wide range of concentrations on the jumping performance of *Drosophila* and compare this with lifespan and anatomy changes. Some of hypotheses may proposed as following, Does a change in diet, sufficient to induce DR, affect muscle performance? Do long-lived flies have stronger muscles than short-lived flies? In DR, is muscle performance maintained at a plateau for longer? What are the impacts of additional food? Can changes in muscle performance be explained by changes in body size or mass?

First, we tested these hypotheses in the standard lab flies, *Canton-S*. Secondly, we have examined the suitability of the Wee-*P* fly as a model in which the muscle performance could be correlated with its size during aging. We find that the *Canton-S*/Wee-*P* cross provides a better model and have therefore tested our hypotheses in this, more robust, outcross.

Chapter 3 Materials and Methods

3.1 *Drosophila* Stocks and Husbandry

Our stocks were wild type *Drosophila melanogaster*: *Canton-S* (CS) or the Wee-*P* 26 transgenic (Clyne *et al.*, 2003). *Canton-S* flies had been maintained at the University of York stocks for > 20 years; Wee-*P* flies for > 5 years at York. Flies were kept in vials at 25 ℃ on standard sugar-yeast-agar medium (Carpenter, 1950, full details in Appendix) and were transferred to fresh vials every 3 to 4 days.

Canton-S/Wee-*P* Crosses: Adult virgin female *Canton-S* flies, less than 8 hours old, were collected on the day of eclosion by CO_2 anaesthesia, and placed in vials containing one of the experimental food types (**Table 2**) and male Wee-*P* 26 flies added. All crosses were also maintained at 25 ℃ and turned over every 3 to 4 days to maintain isolation of parents and offspring.

Table2 Composition of the different experimental food types.

Yeast Concentration	Ratio of yeast in food to normal stock	Composition
1%	Quarter	6.25g yeast/ 50g sugar / 7.5g agar medium
2%	Half times	12.5g yeast/ 50g sugar / 7.5g agar medium
4%	Standard	25g yeast/ 50g sugar / 7.5g agar medium
8%	Two times	50g yeast/ 50g sugar / 7.5g agar medium

*yeast: Allison dried active baking yeast, Westmill Foods Ltd, Maidenhead, UK.

Agar: Agar Technical n.3, Oxoid Ltd, England.

3.2 Lifespan Assay

Larvae were raised on standard food, and adult females collected within 24 hours of eclosion, then placed in vials containing the different food types. For each food treatment we used approximately 50 female flies separated equally in 5 vials and maintained at 29℃. The number of living flies was recorded every 2 days and flies were changed to new vials every 3 to 4 days.

3.3 Anatomy measurements

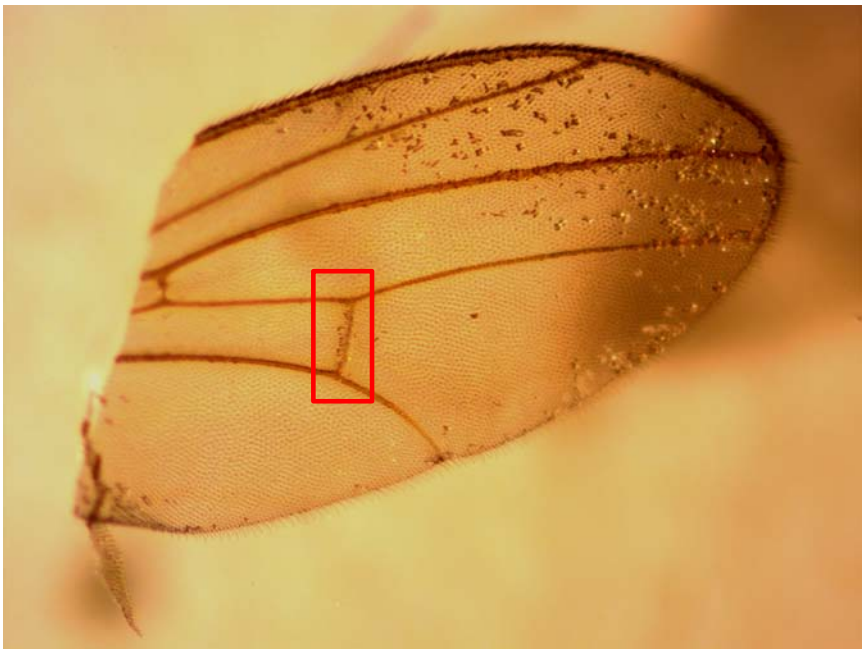
3.3.1 Body Mass Assay

Flies of known age were anaesthetised with ether and placed in a petri dish on a OHAUS Analytical Standard weighing balance to determine the body mass.

3.3.2 Distance between eyes and wing vein

Flies of known age were anaesthetised with ether and observed with a Stemi 2000-C Ziess microscope and AxioCam ERc 5s camera. Photomicrographs (**Figure 5A**) were made of the head and then the wings were cut off and photographed (**Figure 5B**). A calibration bar (1mm) was used as a reference. The actual distance was calculated using Image Processing and Analysis in Java (Image J) (version 1.44i, National Institutes of Health, USA; http://rsbweb.nih.gov/ij/). Data was tabulated in Excel .

A



B

Figure 5

Measurement of the size of flies. (**A**) The graphic shows the length of wing vein (posterior crossvein) as measured in the experiment. (**B**) The graphic shows the distance between eyes as measured in the experiment.

3.4 Jumping Assay

The jump performance of the fly was measured using a flexible beam ergometer (**Figure 6A**) (Harvey *et al.*, 2008). All six legs were free to be placed on the platform at the end of the beam.

All the jumping experiments were only recording female flies because of their larger size, the process of using ergometer was described in (Elliott *et al.*, 2007; Harvey *et al.*, 2008). The flies were anaesthetised with carbon dioxide, and glued the dorsal surface of their thoraces with the tungsten pins on the end of wooden cocktail sticks. After allowing 20-30 minutes recovery, the cocktail sticks holding the fly were mounted on a MM3 micromanipulator, horizontally above the platform, under visual control. The giant descending neurons were then stimulated via electrodes inserted into the eyes, the jumping pathway activated, and then the fly started jumping. The final result was the contraction of the tergal depressor of the trochanter muscle (TDT) through the stimulation of its motoneuron. To find magnitude and direction, the traces were recorded as a variation in the illumination of the photodiode and transformed to a graph using the DasyLab and Dasyview software (**Figure 6B, C**). The changes in vertical and horizontal traces were imported into Excel and calculated using Pythagoras' theorem for magnitude of total displacement and trigonometry for direction of movement (Harvey *et al.*, 2008).

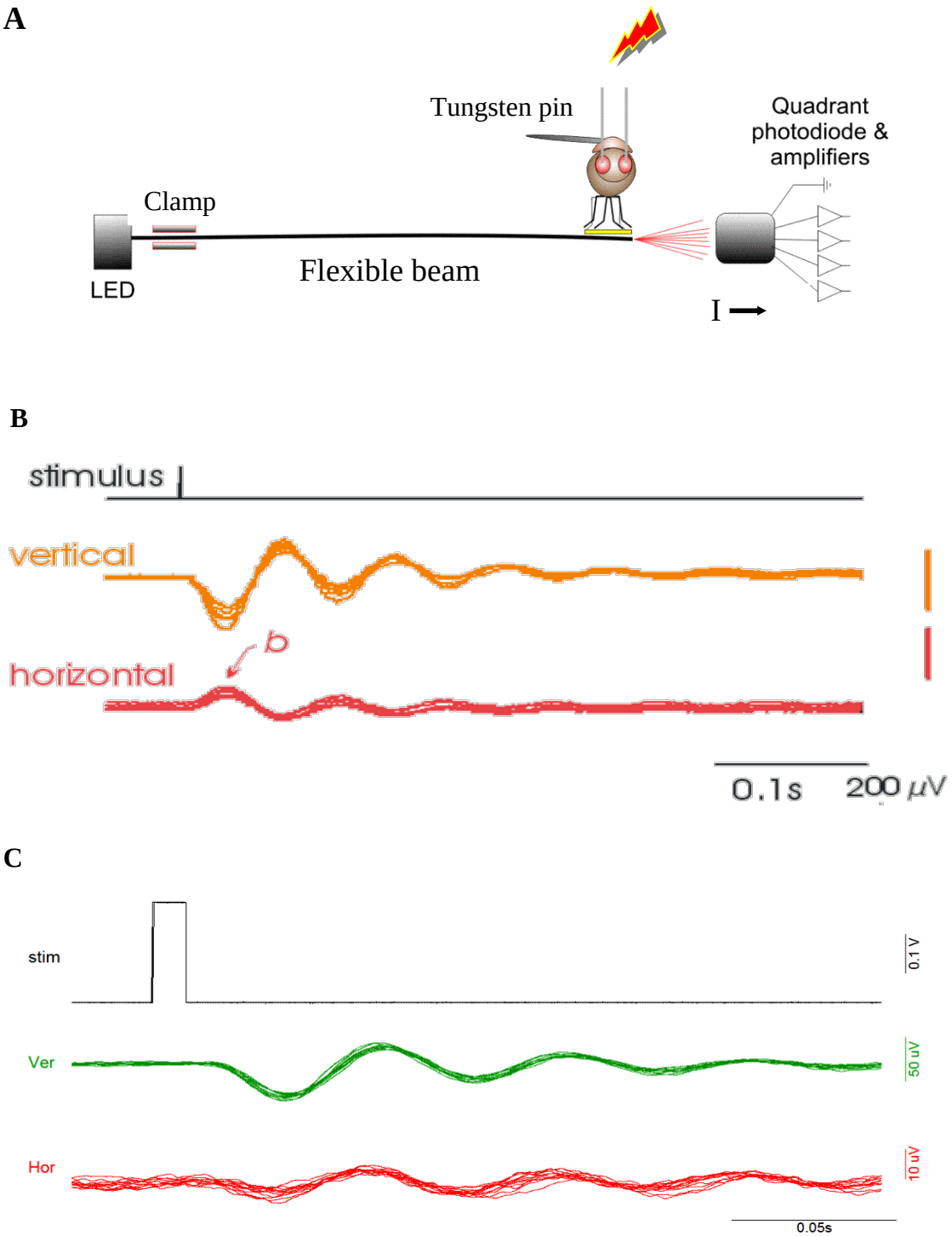


Figure 6

Measurement of the horizontal and vertical movements of the jumping output. (**A**) The ergometer. The fly is glued to a tungsten pin by its thorax at the end of wooden cocktail sticks, mounted with a micromanipulator and placed over the platform (5 X 5 mm) from the end of flexible light beam. When the electrodes stimulation into its head, activated the jumping pathway, then the fly will jump. The platform and the flexible light beam will move and the intensity changes will recorded by four quadrants of the photodiode. (**B**) The data is transferred into computer and generated the graph with vertical and horizontal. Vertical represents upward and downward movement and horizontal means forward and backward movement of platform. (**C**) Six successive responses on a faster timescale, overlaid to show the slight changes in output between responses. (a. modified after (Elliott *et al.*, 2007))

3.5 Statistical Analysis

All data in our experiment were analysed by SPSS statistical software (v, 18.0; SPSS, Chicago, IL). For comparison of only two groups, independent *t*-test was performed to determine the difference between the means of two samples. Multiple significance tests were calculated in ANOVA by using Tukey HSD and Bonferroni methods. Estimates of the survival of lifespan assays were calculated by life table analysis using the Kaplan-Meier method and survival curves compared with use of the Log-rank, Breslow and Tarone-Ware tests. Statistical significance was defined as a *p*-value < 0.05. All error bars represent Mean±SEM.

Chapter 4 Results

4.1 How does feeding *Canton-S* flies with standard food affect the jump performance

To observe how muscle output decreased during the aging, jumping performance was measured at 6 different time points, 3, 7, 14, 21, 28, and 35 days, using an ergometer.

The general trend followed by all the flies in standard food was a significantly decreased in jumping performance with age. The differences between the time points are significant (ANOVA - ***$p < 0.0001$) (**Figure 7**).

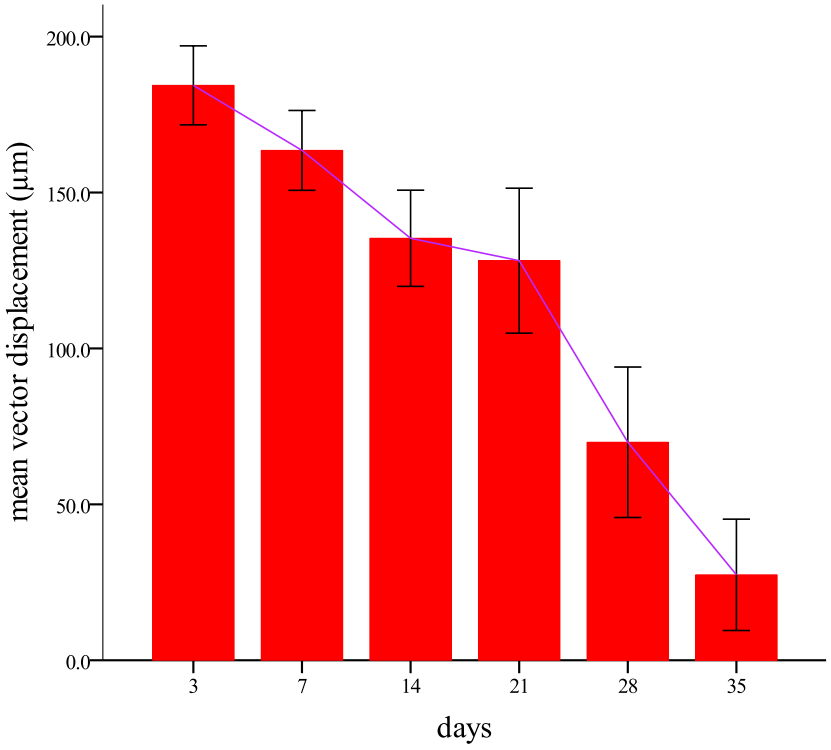


Figure 7

On standard food, jumping performance declines steadily with age. The graph shows the mean and SE for each time point, with at least 15 flies in each sample. Analysis of variance shows that the difference is significant (F 5,89 df = 18.0, $p < 0.0001$). (Error bars: ± standard error of the mean)

4.2 How does jump performance of Wee-*P* compare with *Canton-S* flies

To determine if the Wee-*P* flies were a suitable model with good jumping performance, and to observe muscle output in different types of flies, jumping performance was measured with 2 different genotype of flies, *Canton-S* and Wee-*P*.

Wee-*P* flies showed very significantly less jump muscle output (about 50% less) (independent *t*-test - ***$p < 0.0001$) (**Figure 8**). Therefore, in order to collect data from flies with fluorescence expressed in the TDT muscle, we tested the cross between *Canton-S* and Wee-*P* flies.

The jumping performance of *Canton-S*/Wee-*P* showed no significant difference from the *Canton-S* flies (**Figure 9**), with a mean 126.39 μm ± SE 5.9.

To test for a physical anatomical difference and for comparison with jump performance, body mass, distance between eyes and length of wing vein were measured with 3 different genotypes of flies in 3 day old virgin female flies. Wee-*P* flies showed very significantly lower average body mass compared to *Canton-S* and *Canton-S*/Wee-*P* flies (ANOVA - ***$p < 0.0001$) (**Figure 10A**); however, the distance between eyes and length of wing veins, were only slightly different between Wee-*P* and *Canton-S*/Wee-*P* flies (ANOVA - *$p < 0.05$). There was no difference between *Canton-S*/Wee-*P* flies and *Canton-S* flies (**Figure 10B and C**).

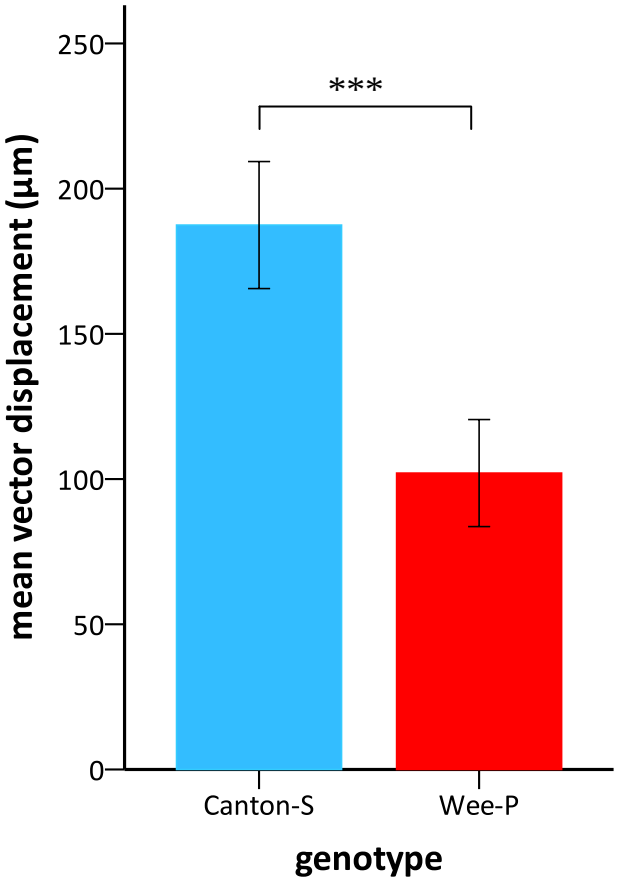


Figure 8

Wee-*P* flies are not a suitable model as they do not jump well. Levene's Test for the equality of variance gives a *p*-value > 0.05, so equality of variance can be assumed. Next, at the $\alpha = 0.05$ level of significance, there is enough evidence to conclude that there is a difference between *Canton-S* and Wee-*P* flies in jump performance. (Error bars: ± standard error of the mean)

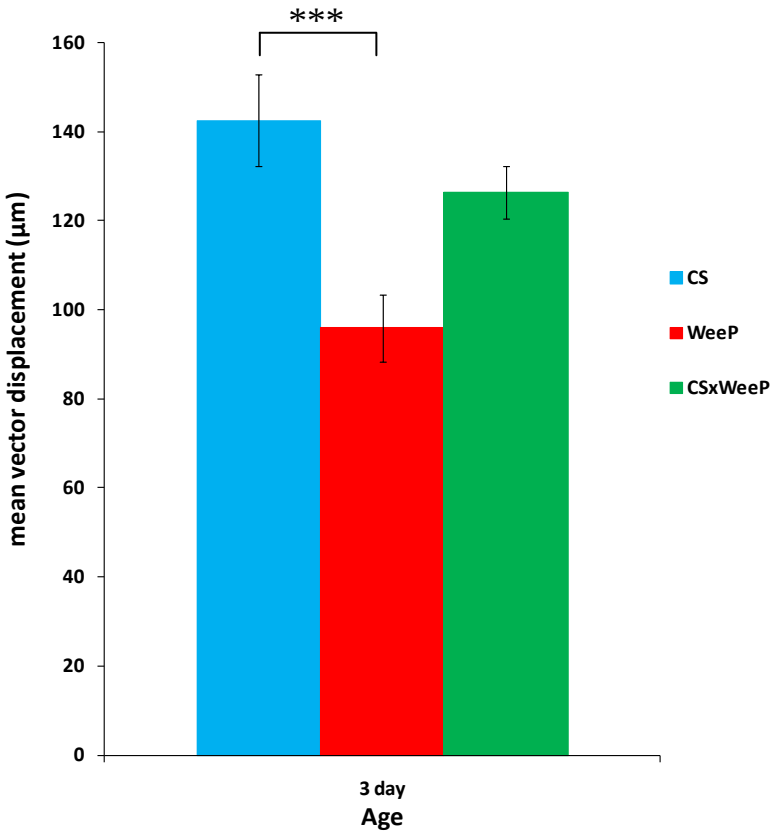
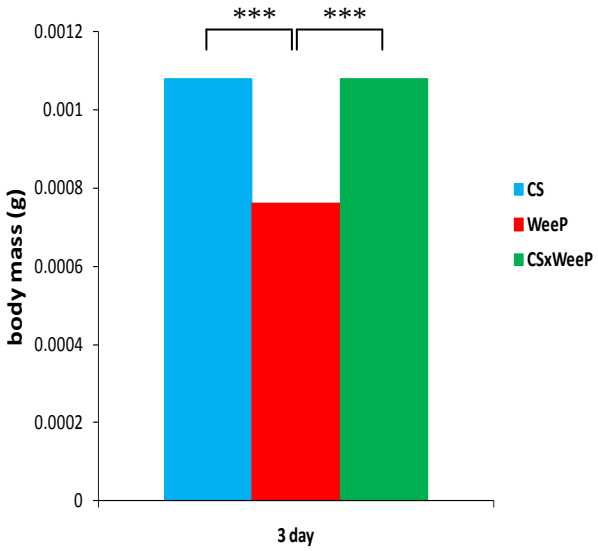


Figure 9
Jumping performance between different fly genotypes. Mean jumping performance with *Canton-S*, Wee-*P*, and *Canton-S*/Wee-*P* 3 day-old virgin female flies. There is no significant difference with *Canton-S*/Wee-*P* and other two fly genotypes, and significant difference between *Canton-S* and Wee-*P* flies. (Error bars: ± standard error of the mean)

A



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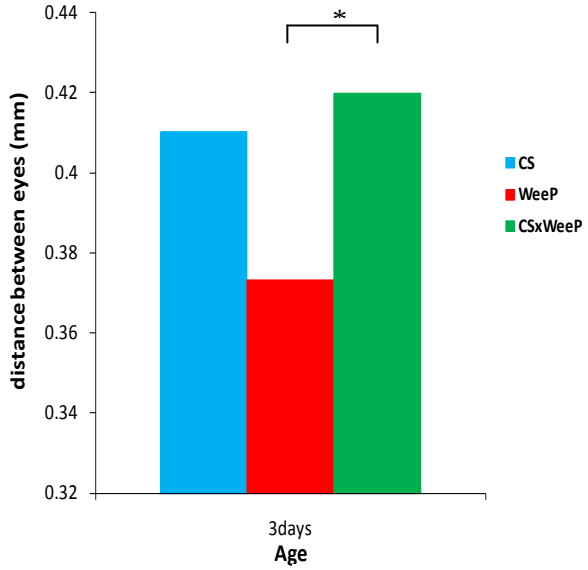

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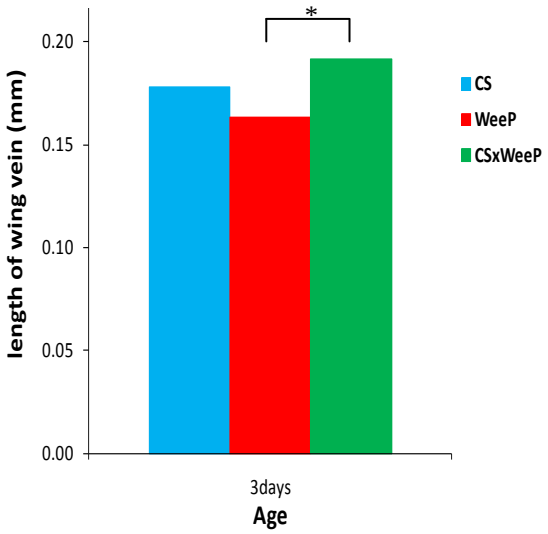


Figure 10

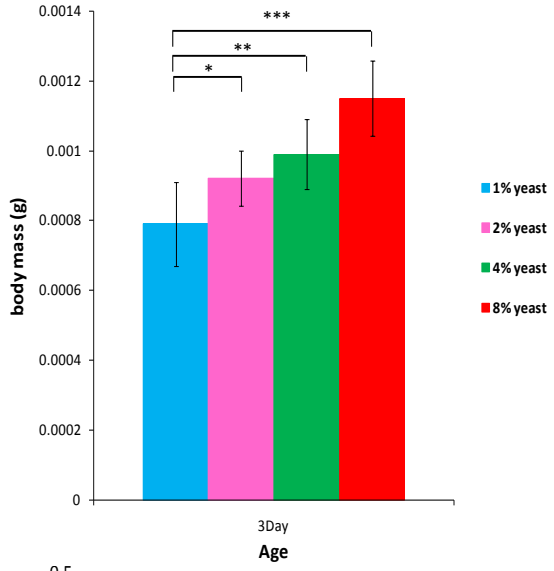
Body mass, distance between eyes, and distance between wing vein of 3 day old female flies with 3 different genotypes. (**A**) The body mass of Wee-*P* flies is different from both *Canton-S* and *Canton-S*/Wee-*P* flies. (**B**) There is slight difference between Wee-*P* and *Canton-S*/Wee-*P* flies on distance between eyes. (**C**) There is only a small difference in the length of the wing vein between the 3 genotypes. (Error bars: ± standard error of the mean)

4.3 How does feeding larvae with different yeast concentration affect their size and jump performance?

To observe the anatomical changes in *Canton-S*/Wee-*P* flies fed with difference concentration of yeast from larval stage, we measured the body mass, distance between eyes and length of wing vein of 3 day old virgin female flies. Overall, feeding the larvae with increasing yeast correlated with increased size of the adult fly at 3 days old. The biggest effect was on body mass, where the difference between 1% and 8% yeast was 50%, and the smallest effect was on the length of the posterior crossvein, where the difference was only 14%. For body mass, flies fed with 1% yeast showed little significant difference with 2% yeast, significant difference with 4% yeast, and very significantly with 8% yeast (ANOVA - *< 0.05; **< 0.001; ***p < 0.0001) (**Figure 11A**). However, for distance between eyes and length of wing veins, former measurement only showed slightly difference between 1% yeast and high concentration groups (4% yeast and 8% yeast) (ANOVA - *p < 0.05) and the latter measurement did not show any difference between each yeast concentrations (**Figure 11B and C**).

We also compared the jumping performance of 3 day old adults which had eclosed from larvae raised on food with different yeast concentrations. Here we found the best jumping performance was from those treated with 4% yeast. This was approximately twice the jump performance of those raised on 1% yeast, and nearly 7% of those raised on 8% yeast. The jumping performance of *Canton-S*/Wee-*P* flies which were raised in different percentage of yeast food treatment were statistically having slightly difference between 3 groups-1%, 4%, 1%, 8% and 2%, 4% as shown by Tukey HSD and Bonferroni tests (AVOVA *p < 0.05; **p < 0.01) (**Figure 12**) .

A



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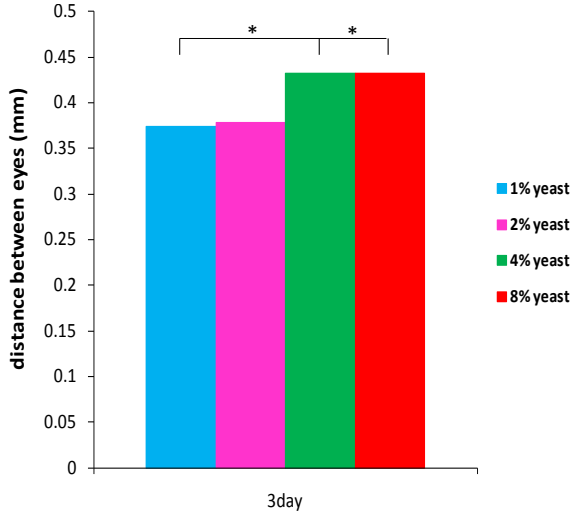

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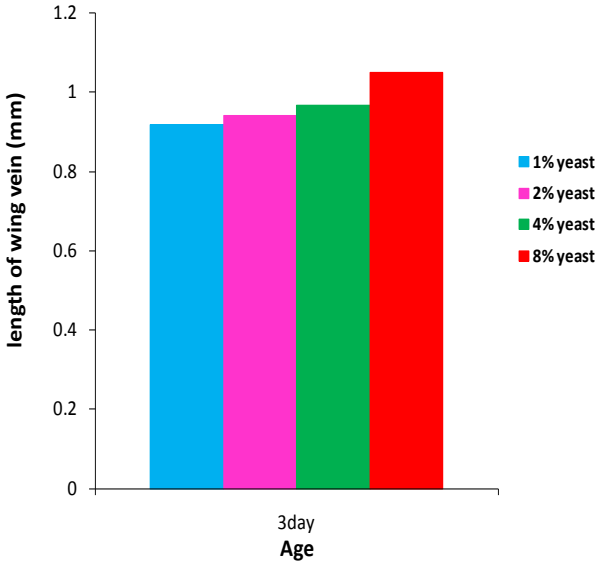


Figure 11

Body mass, distance between eyes, and length of wing vein (mm) of 3 day old *Canton-S*/Wee-*P* flies raised from larval stage in different concentration of yeast food treatment. (**A**) The graph shows significant difference between different concentrations, highest concentration had highest body mass and decreased by concentration. (**B**) The graph shows slightly significant difference between lowest concentration and high concentration groups on distance between eyes. (**C**) The graph shows there is no any significant difference in any yeast concentrations with length of wing vein.

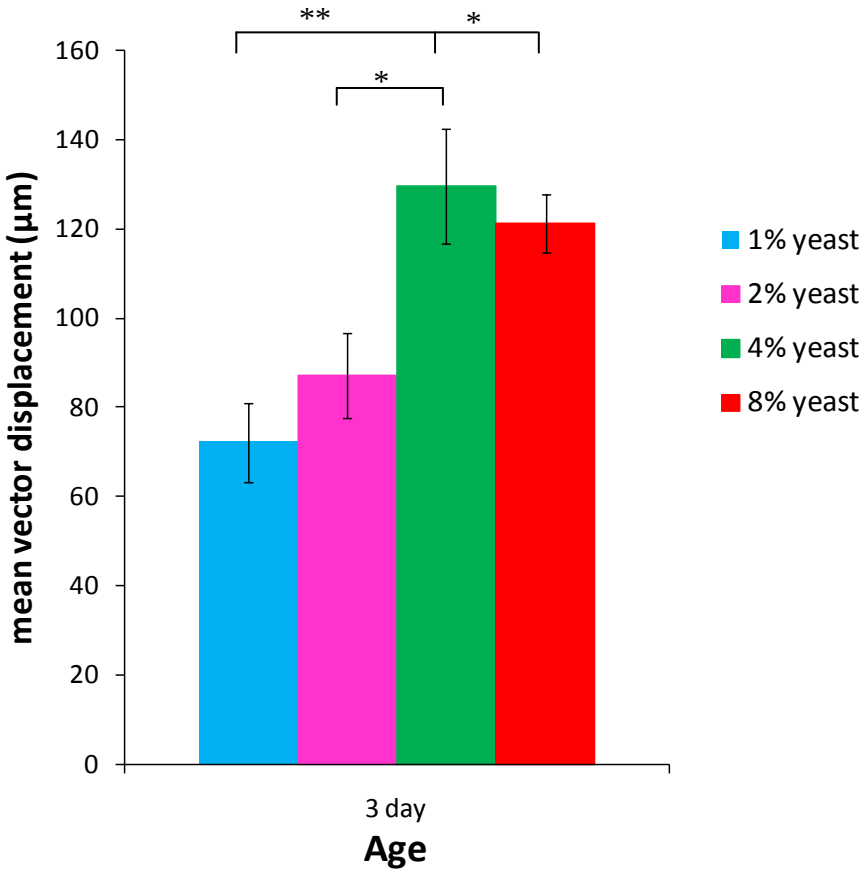


Figure 12

The yeast composition of larval food affects jump performance of young (3 day old) flies (*Canton-S*/Wee-*P*). The Tukey HSD tests suggest that the 1% differs from both the 4% and 8% treatment (p= 0.003, $p < 0.05$ respectively) and 2% slightly differs from 4% treatment ($p < 0.05$). However, the 1% and 2% treatments are not significantly different ($p = 1.000$). The same *P* values were obtained in the Bonferroni post-hoc tests. (Error bars: ± standard error of the mean)

4.4 How does yeast concentration affect the lifespan?

To compare if the different percentage of yeast food treatment had an effect on the survival/mortality of *Canton-S* and *Canton-S*/Wee-*P* flies, flies were fed with food containing different percentages of yeast (**Figure13A, and B**). The Kaplan-Meier statistical analysis with three different tests, Log-Rank, Breslow, and Tarone-Ware tests (***$p < 0.0001$ in all tests) in SPSS, showed significant differences in longevity between high concentration (4% and 8%) and low concentration (1% and 2%) in *Canton-S* and significant difference between each concentration in *Canton-S*/Wee-*P*. On the whole, flies survived well in both *Canton-S* and *Canton-S*/Wee-*P* flies- in *Canton-S*, the survival curve remained relatively flat until 20 days in 4% and 8% yeast, but did better (30 days) in 1% and 2 % yeast; in *Canton-S*/Wee-*P*, the overall lifespan are longer compared to *Canton-S*. The survival curve remained nearly flat until 29 days in general. Flies survived best on 2% yeast in both groups, with median age-34 and 47 days; either lower or higher concentrations were detrimental to survival with the median age being reduced to between 24-32 days in *Canton-S* and 36-41days in *Canton-S*/Wee-*P* (**Figure 13C)**.

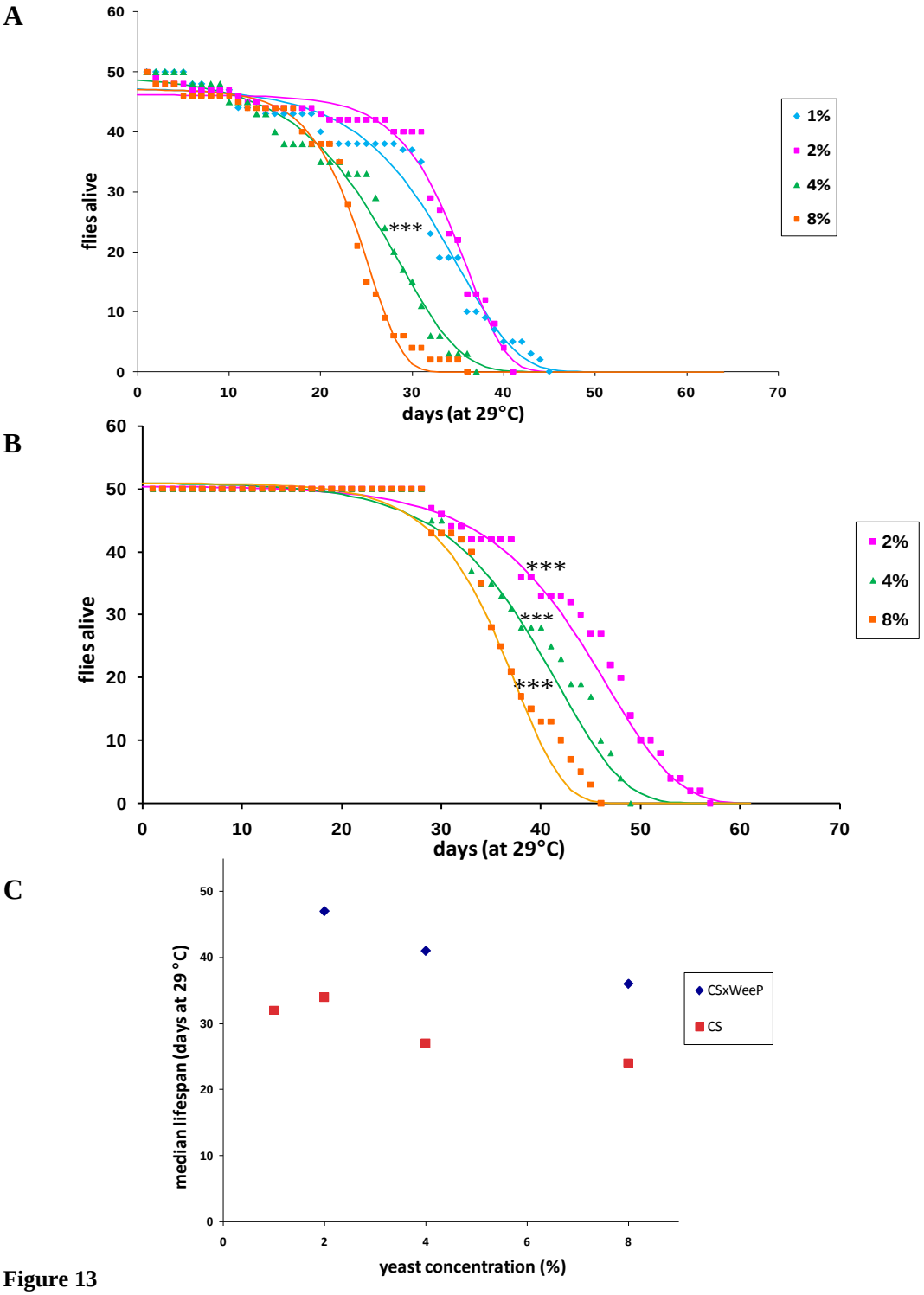


Figure 13

Lifespan curves for the media with different percentage of yeast in *Canton-S* and *Canton-S*/Wee-*P*. (**A.**) The graph shows the number of remaining *Canton-S* flies with different percentage of yeast food treatment by days. (**B.**) The graph shows the number of remaining *Canton-S*/Wee-*P* flies with different percentage of yeast food treatment by days. By using Kaplan-Meier analysis includes 3 different tests (Log-Rank, Breslow and Tarone-Ware tests to compare the lifespan distribution showed a significant difference between the curves with ****p*-value < 0.0001 and df=1. **(C.)** The graph shows the median lifespan as a function of yeast concentration in SY media in *Canton-S* and *Canton-S*/Wee-*P* flies.

4.5 How does feeding the flies with different yeast concentration affect their size and jump performance?

To observe the difference between anatomy change and jumping performance of *Canton-S*/Wee-*P* flies in different yeast food concentration at different ages, larvae were raised on 4% yeast (standard lab food) and transferred to different food on the day of eclosion. Their body mass was measured with 3 time points-3, 28, and 35 days. Even by 3 days, there is a significant correlation between yeast concentration and body mass, with the adults on 8% yeast weighing 44% more than those on 2% yeast. The mass continued to increase with age, with the flies on 8% yeast gaining most mass. There was no sign of loss of mass in old flies. The body mass measurement showed statistically significant difference either between ages or concentrations in *Canton-S*/Wee-*P* flies (ANOVA - ***$p < 0.0001$) (**Figure 14**).

A comparison between the age of *Canton-S* and *Canton-S*/Wee-*P* flies fed by different percentage of yeast, and the jumping performance was measured at 6 different time points can help us understand if the DR affect the jumping performance of flies differently with age, using an ergometer.

In general, *Canton-S* flies maintained in different yeast food treatments had a similar decline in jump performance. There was a statistical difference between lowest concentration (1%) and the highest concentration (8%) as shown by Tukey HSD and Bonferroni tests. The rest of yeast food treatment (2% and 4%) did not show significant differences (**Figure 15**). However, the data from the flies feed with 8% yeast are out of line at 21 days; well below the rest of the data samples, but by 28 days the lines have converged again. When the 21 day data were removed from the results, there was no significant difference between the yeast food treatments. We believe that the 21 day, 8% yeast data point is aberrant [possibly due to a sudden variation in the incubator temperature].

With the *Canton-S*/Wee-*P* flies, the jump performance also declined gradually over the experimental period, with little difference between the different yeast food treatments. The Post-hoc tests showed significantly difference between two groups- 2% & 4% and 4% & 8%, but there was no significant difference between 2% and 8% (**Figure 16**). Since the lowest and highest yeast treatments (2% and 8%) do not differ significantly, we conclude that the overall effect is not biologically important. Thus both our experimental genotypes, the inbred *Canton-S* and the outcross *Canton-S*/Wee-*P* lead to the same general conclusion, that DR does not have a big impact on jumping performance.

To confirm this conclusion, we compared the median lifespan (i.e. the time for 50% of the flies to die), the time to 50% of the initial jump performance and the time taken for 50% gain in body mass at each yeast concentration (**Figure 17**). As expected, the 2 % yeast food treatment has the longest lifespan in both fly genotypes. However, the jumping performance showed much less dependence on yeast concentration than lifespan and body mass gain. This is true for both *Canton-S* and *Canton-S*/Wee-*P* flies.

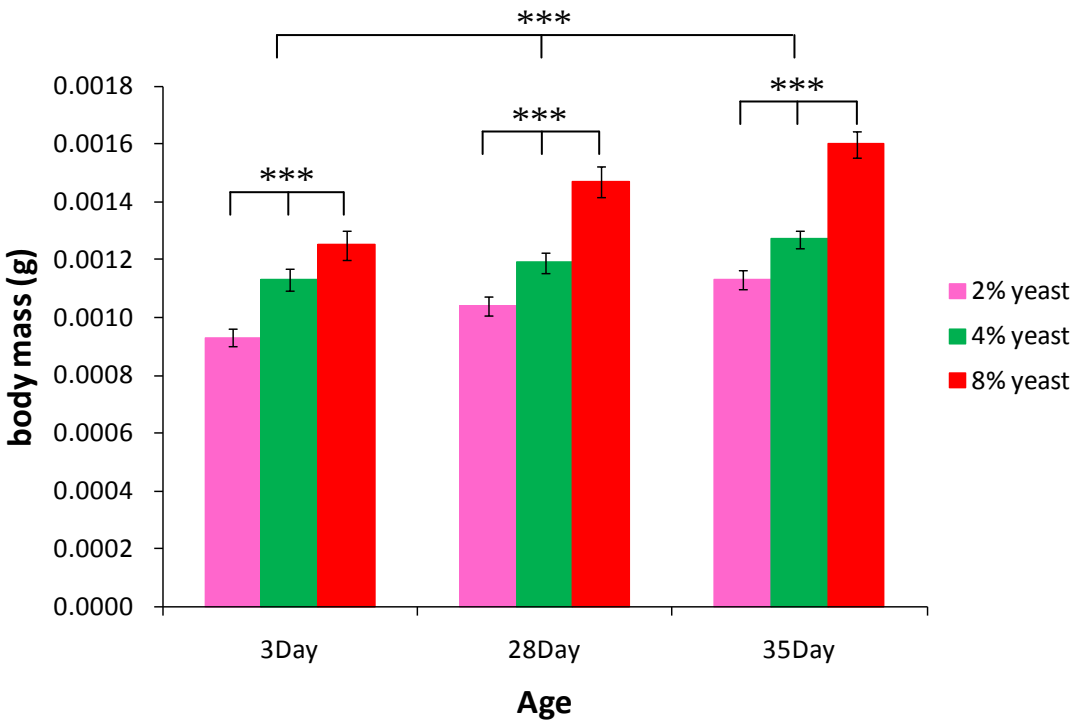


Figure 14

Body mass of *Canton-S*/Wee-*P* in different concentration of yeast food treatment at different ages. The graph shows significant difference between different concentrations at different ages. (Error bars: ± standard error of the mean)

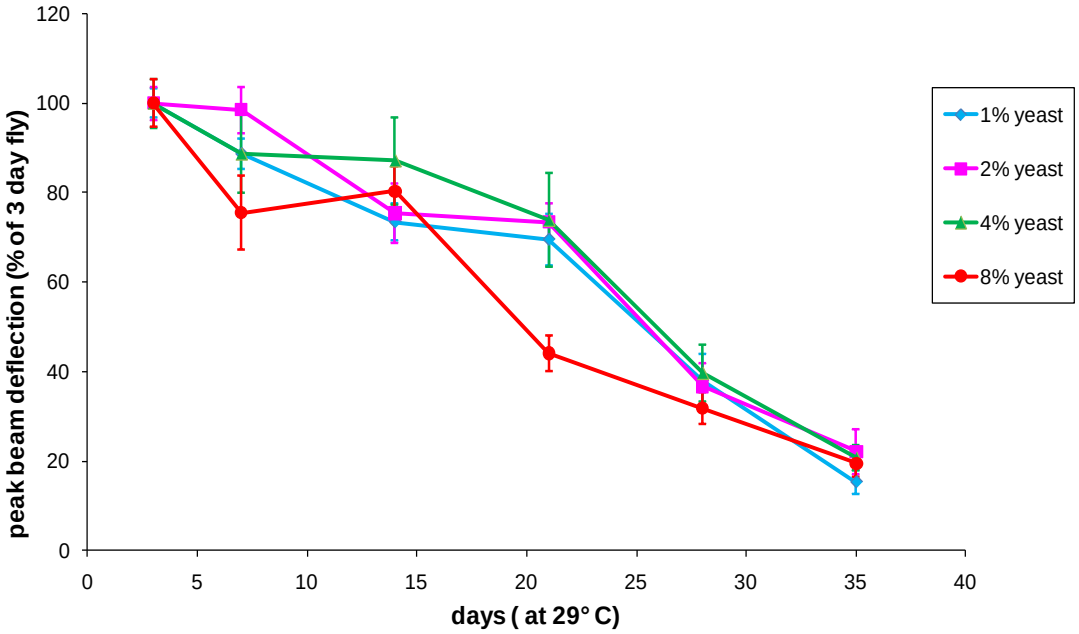


Figure 15

Jumping performance does not decline very differently when the concentration of yeast is varied (*Canton-S* flies). The Tukey tests suggest that the 1% differs from 8% treatment ($p < 0.05$). However, 1% and 2%, 8% treatments are not significantly different (both p=1.000). The same P values were obtained in the Bonferroni post-hoc tests. (Error bars: ± standard error of the mean)

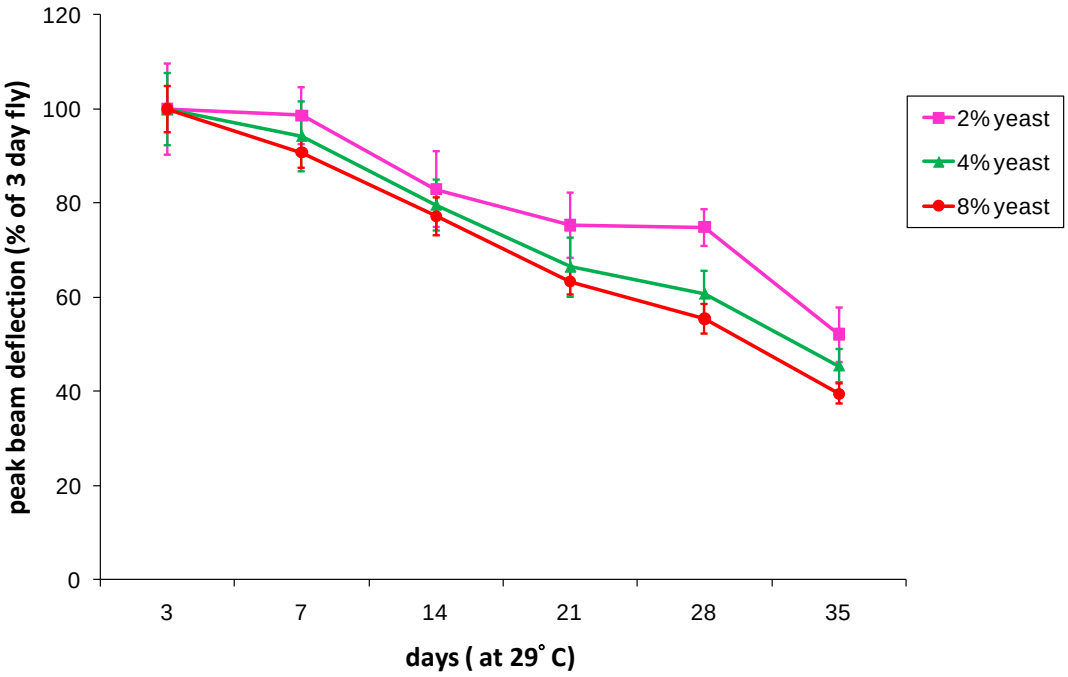


Figure 16

Jumping performance does not decline very differently when the concentration of yeast is varied (*Canton-S*/Wee-*P* flies). The Bonferroni post-hoc tests suggest that the 4% differs from both the 2% and 8% treatment (p <0.001 and p= 0.002 respectively). However, the 2% and 8% treatments are not significantly different (p = 1.000). The same P values were obtained in the Tukey tests. (Error bars: ± standard error of the mean)

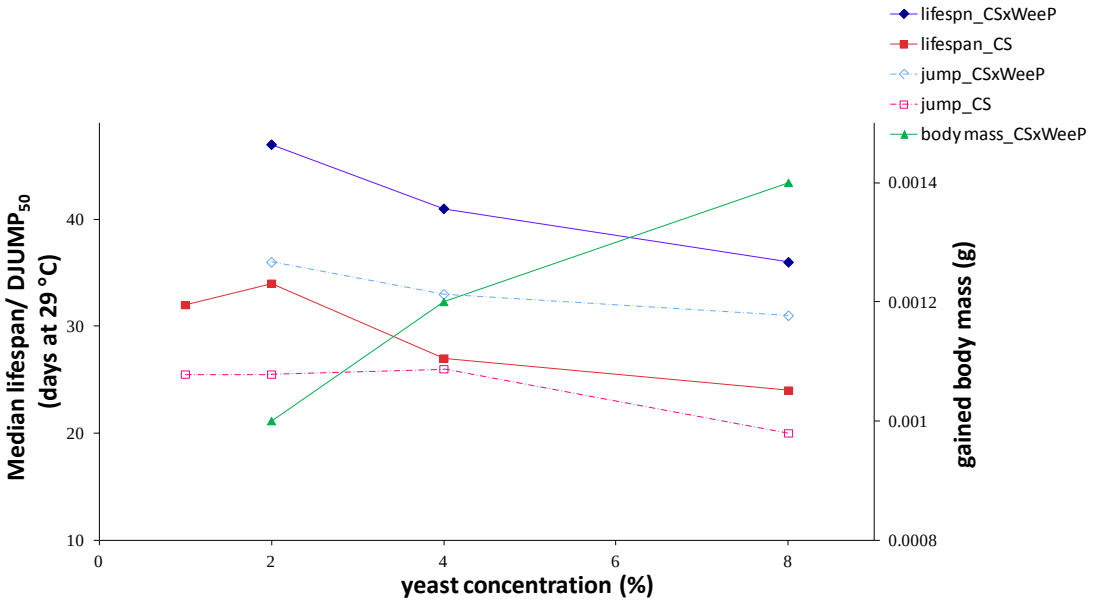


Figure 17
Dietary restriction has more effect on lifespan and the median gain in body mass than jump performance in *Canton-S* and *Canton-S*/Wee-*P* flies. The median lifespan decreased as the yeast concentration was increased, and 2% yeast concentration produced the optimal lifespan. We define $DJUMP_{50}$ as the age at which the jump performance reaches half the initial jump performance. $DJUMP_{50}$ is not significantly affected by different yeast concentration in *Canton-S* or *Canton-S*/Wee-*P* flies.

Chapter 5 Discussion

The main finding from these experiments is that dietary restriction (DR) does not affect the jump muscle performance, although it affects lifespan and body mass. On the other hand, over-feeding flies results in a shorter lifespan and also a quicker decline in jumping performance compared to the flies on standard food. We can thus begin to answer the questions posed in the Introduction:

- Does a change in diet, sufficient to induce DR, affect muscle performance? No.
- Do long-lived flies have stronger muscles than short-lived flies? No.
- In DR, is muscle performance maintained at a plateau for longer? No.
- What are the impacts of additional food? Reduced lifespan and with no biologically significant decline in muscle performance.
- Can changes in muscle performance be explained by changes in body size or mass? This question is not fully answered.

However, is the apparatus sensitive enough to resolve changes in muscle performance, due to changes in (i) size or (ii) feeding? The first issue was addressed by comparing the *Canton-S*, Wee-*P* and their heterozygote genotypes, the second by examining the morphology and jump ability of young adults, which had been raised as larvae on different yeast diets.

5.1 How does size and jump performance of Wee-*P* compare with *Canton-S* and *Canton-S*/Wee-*P* flies?

The jumping performance of Wee-*P* flies was 50% less than *Canton-S* flies at age 3 days (**Figure 8**). This results in a much worse signal to noise ratio, making the measurement (especially at old age) much worse. Wee-*P* flies are therefore not a suitable model for monitoring jumping performance.

Poor jumping performance in the Wee-*P* could result from the GFP insert producing a defect in the actomyosin ATPase, a defect in the sliding filaments or be a simple consequence of a smaller muscle volume. Measurements of indicators of body size (wing vein length, eye separation and mass) shows that the Wee-P flies are much smaller than *Canton-S* flies (**Figure 10**) The ratio of mass/jump performance is the same in *Canton-S* and Wee-*P* flies as would be expected if the TDT muscle always occupies the same proportion of the volume of the fly.

The heterozygote of *Canton-S* and Wee-*P* flies had the same size and jumping performance as the *Canton-S* but still have the GFP, so we can discount any effect of the GFP on the ATPase or sliding filaments.

Taken together, these factors suggest a homeostatic mechanism, whereby the fly tries to regulate its overall muscle output to the required level; so that it can take of and start flying away from predators affectively.

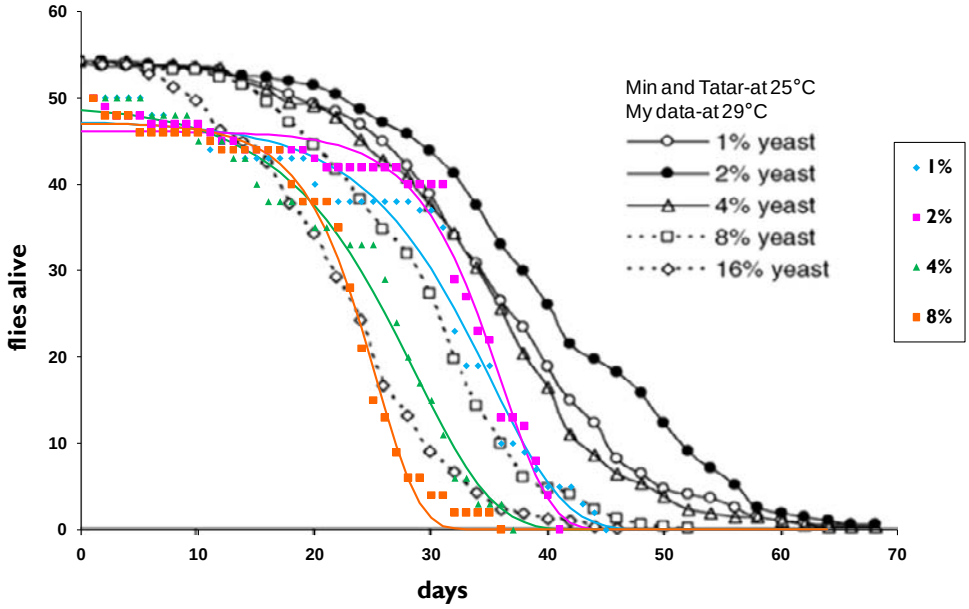
5.2 How does feeding larvae with different yeast concentration affect their size and jumping performance?

When larvae were grown on different diets, there was little change in average body size (length of wing vein, not significant, eye separation < 15%) of 3 day old heterozygote flies in different yeast concentration; however, there was a large difference in mass (~45%) and jumping performance (79%). This shows that the apparatus is sensitive enough to record differences in jumping performance, even in flies of similar size. However, when fed with more yeast than normal, the body mass increased but the jump performance decreased. We conclude that body size is not the only determinant of jumping performance and that measurement of muscle size is crucial.

5.3 How does yeast concentration effect the lifespan in *Canton-S* and *Canton-S*/Wee-*P* flies

As there has been debate in the literature about the best media to demonstrate lifespan extension by DR (Bourg and Minois, 2005; Bass *et al.*, 2007), we can validate our lifespan data by comparison with that from the Tatar and Partridge groups. Our differences in *Canton-S* lifespan compare well with data from corn-sugar-yeast food (Min and Tatar, 2006). From their figure 1, the extension of *Canton-S* lifespan by DR was at the similar level compared with our food manipulations (**Figure 18A and B**). For example, we found a 16% change in lifespan from 4% to 1% yeast, and Min and Tatar found a 5%. Moreover, in (Partridge *et al.*, 2005), figure 1, the lifespan of female *Drosophila* on sugar-yeast food was longer at 2% than 1% yeast level (**Figure 19**), which is also similar to our result. The data from Min and Tatar, Partridge *et al* and our experiments all showed flies with DR food treatment (reduced yeast concentration) have longer lifespan compared to living in standard or higher yeast concentration and flies have longest lifespan in about 2% yeast concentration food from all results. However, in all of their experiments, lifespan was longer than in ours. This may due to the different experimental temperature (25℃ with Min and Tatar and 29℃ with our experiments) (Mair *et al.*, 2003), rearing conditions and procedure for food preparation. As we had expected, the *Canton-S*/Wee-*P* heterozygote lived longer than the *Canton-S*, with no mortality before 30 days, and the median lifespan in 2% yeast was 38% longer. These may possible caused by a genetic difference in response to variation in yeast concentration.

A



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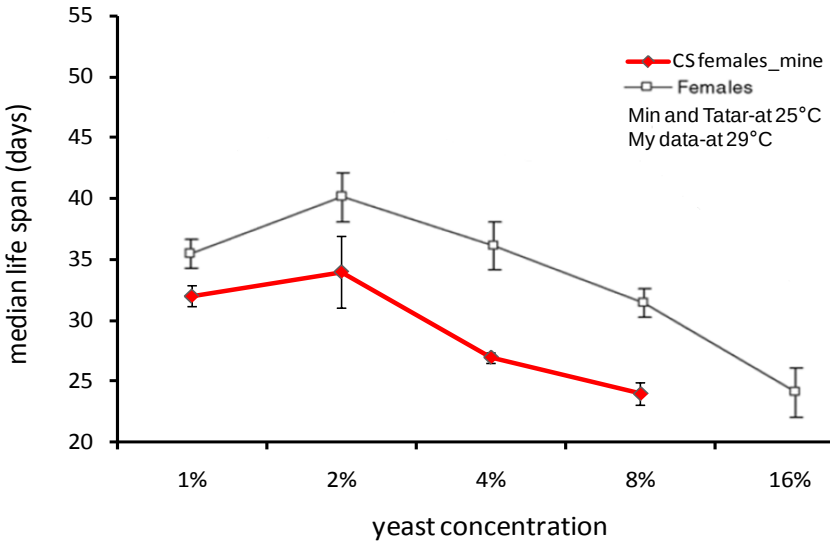

Figure 18

Comparison lifespan of *Canton-S* between our data in colour and that reported by Min and Tatar in black and white. **(A)** The graph showed the *Canton-S* female lifespan with different concentration of yeast food treatment calculated by days. **(B)** The graph shower the median lifespan in different concentration of yeast food treatment, compared with our *Canton-S* median lifespan. (Modified after (Min and Tatar, 2006))

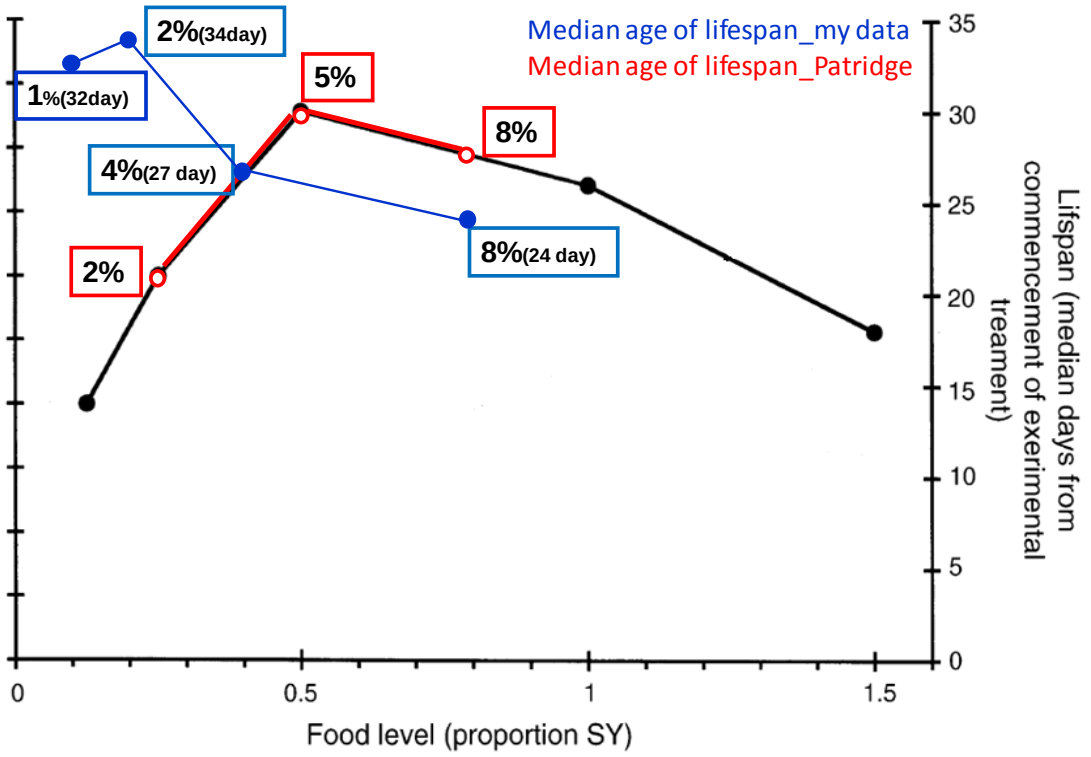

Figure 19

Median lifespan in different concentration of yeast food treatment, compared between our *Canton-S* median lifespan in colour and that reported by Partridge *et al*. in black and white (Modified after (Partridge *et al.*, 2005))

5.4 How does aging on standard food affect the jumping performance in flies

Overall, the jumping performance of *Canton-S* flies fed with standard yeast-sugar food showed significant decrease as the flies aged (**Figure 7**). There is no sign of the 2 week optimal plateau suggested by initial experiments (Harvey *et al.*, 2008); the best performance is at 3 days and by 7 and 14 days the gradual decline is already underway. The same consistent decline starting at 3 days is seen in the *Canton-S*/Wee-*P* outcross. The declined of heterozygote is slower than the inbred strain, and it refers to our data (**Figure 16**). The potential explanations for this decline include a loss of muscle mass or a reduction in muscle effectiveness, possibly related to mitochondria dysfunction or changes in contractile proteins. In *Drosophila* flight muscle, accumulated structural damage in mitochondrial is suggested to cause a drop in ATP levels and so affect the flight ability of flies age (Miller *et al.*, 2008). Flies appear to compensate for this by increasing the power output of the individual muscle fibres (Miller *et al.*, 2008). However, in humans and other vertebrates, a reduction in the contractile properties of muscle was considered more important (Larsson *et al.*, 1997; Krivickas *et al.*, 2001; Lowe *et al.*, 2001, 2002; Prochniewicz *et al.*, 2005; Ochala *et al.*, 2006, 2007; D'Antona *et al.*, 2003, 2007; Yu *et al.*, 2007). From our results, there is no sign of nerve conduction failure in older flies, unlike Angeles (2009), suggesting neurodegeneration is not a key contributor of the decline jump performance.

5.5 How does dietary restriction affect jumping performance?

Although DR has a clear affect on both lifespan and body mass, there is no clear effect on jump performance either in *Canton-S* or *Canton-S*/Wee-*P* flies (**Figure 17.**). This is also unlike our manipulations of larval food, which had a big impact on the adult jump performance. The sensitivity and reproducibility of the apparatus suggests that the maximum effect of DR on muscle performance must be < 10%, and therefore, we find no evidence of a change in muscle performance with DR, which might indicate a diversion of resources from reproduction to somatic tissues

Also, interestingly, in a second dietary manipulation, both wild-type flies and those carrying a *PTEN-induced putative kinase 1* (*PINK1*) mutation (Clark *et al.*, 2006; Park *et al.*, 2006; Gautier *et al.*, 2008; Gispert *et al.*, 2009) fed with α-tocopherol treatment have improved lifespan but no change in jumping performance (Xu, 2011). This is similar to our results with yeast dietary restriction treatment. Although this data set was smaller than our DR experiment, it too suggests that muscle performance and lifespan are not tightly coupled.

Initial work with DR suggested a trade-off between reproduction and survival. However, more recent studies have questioned this relationship, providing varying results. Data suggesting a trade off include manipulations of the genes *chico* (Clancy *et al.*, 2001; Tu *et al.*, 2002) and *insulin-like receptor* gene (*dInR*) (Tatar *et al.*, 2001). These are part of the insulin signalling system in *Drosophila*, where the lifespan was promoted but fecundity and fertility reduced. In contrast, *Indy* and *ecdysone receptor* (*EcR*) mutant flies showed increased lifespan and also showed greater fecundity and fertility than control flies (Marden *et al.*, 2003; Simon *et al.*, 2003). Also, in *C. elegans*, *age-1* and *daf-2* are long lived mutants without reduction in reproduction (Flatt, 2011). There are some other studies suggesting evidence for the crucial role of

physiologically based trade-offs and the hormonal controls. For example, high reproduction is related with poor moult, reduced immunological status, decreased investment in sexual ornamentation in birds, decreased growth in guppies, flight capability in crickets and longevity in insects (Shanley and Kirkwood, 2000).

In our experiments, flies in DR have no effect in sarcopenia; however, rats under calorie restriction have less severe sarcopenia compared to normal fed rats (McKiernan *et al.*, 2004).

5.6 How does dietary supplementation affect jumping performance?

The 8% yeast produced the largest increase in body mass without any increase in body size, and lifespan shortened by 11 % and 12 % in *Canton-S* and *Canton-S*/Wee-*P* respectively. Body mass increases are associated with reductions in lifespan in insects and flies organisms (Warbrick-Smith *et al.*, 2006; Kolss *et al.*, 2009). Although the statistical tests showed some differences between 8% and the other yeast treatments at some time points, the overall conclusion is that this is not biologically significant. Thus both the reduction and increase in yeast concentration have a marked effect on lifespan but not on the rate at which jump performance declines with age.

Chapter 6 Conclusion

The main finding from these experiments is that in flies dietary restriction (DR) extended the lifespan, and reduced body mass but had little effect on the jump performance of flies. Even though predator escape is a crucial part of survival, we find no evidence that resources may be diverted from reproduction to muscle, a somatic tissue, to maintain survival. On the other hand, over-feeding flies both shortened their lifespan and led to a more rapid decrease in their jumping performance, compared to the flies on standard food.

If the TDT jump muscle is taken as a typical muscle, then a gradual deterioration in performance with increasing age might be expected in all muscles. This might lead to a slower ability to find food and to ingest it, reducing energy intake and with the consequence of further weakening throughout the organism. We suggest a homeostatic mechanism may be a brake on this vicious circle and further analysis of the size and metabolic profile of the TDT muscle may provide novel insights into the aging process.

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Author's declaration

I hereby certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication.

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Abbreviations

AMP : adenosine monophosphate-activated protein

COX : cytochrome c oxidase

CR : Caloric Restriction

CSA : cross-sectional area

DR : Dietary Restriction

FOXO: forkhead box

GAS : gastrocnemius muscle

GDN : giant descending neuron

GFP : green fluorescent protein

IFM : indirect flight muscle

ImageJ : Image Processing and Analysis in Java

mtDNA : mitochondrial DNA

MQ : muscle quality

MHC : myosin heavy chain

PINK1: PTEN-induced putative kinase 1

PSI: peripherally synapsing interneuron

ROS : reactive oxygen species

SOD : Superoxide dismutase enzymes

Sol : Soleus muscle

TTM : tergal trochanteral muscle

TDT : tergal depressor of the trochanter

TDTMn : tergal depressor of trochanter motoneuron

VNC : ventral nerve cord

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Appendix

Procedure of fly food