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Time restriction feeding as a strategy to reverse the effects of diet induced obesity: Morphology and behaviour

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Abstract

Background: High fat diet induced obesity is the most challenging health problem which leads to other comorbidities worldwide. Obesity is a disorder either due to high calorie diet, circadian disruption or sedentary lifestyle. High fat diet (HFD) resulted in increased obesity and needs urgent solutions to overcome health issues.

Objective: The present study is an attempt to understand the importance of time-restriction feeding (TRF) to reverse HFD-induced obesity. The current study aims to investigate the adverse effects of a high-fat diet on odd-time eating and high-fat diet with that of time-restriction feeding.

Material Method: To address this issue, Drosophila melanogaster were chosen flies that were fed with different food regime such as high-fat diet (HFD), a normal diet (ND) as a control, HFD+TRF and ND+TRF. The assays were used to detect obesity, body weight, size and lipid profile. The locomotor, longevity and CAFÉ assay were performed to access the effect of TRF in obesity related activity.

Conclusion: In results it was found that, TRF has an effective response to obesity by reducing body weight and adiposity caused by a high-fat diet. TRF diet also improves the muscle activity and overall body metabolic function, reflecting in the reduced body weight. These results indicate the efficacy of TRF regimen might be a potential novel non-pharmacological strategy to reverse the high fat diet induced obesity.

Keywords: obesity, time restricted feeding, high fat diet, *Drosophila melanogaster,* morphometry

Introduction

Technological advancements have increased the pace of life to several folds, however, it has also made human beings physically inactive and lack of physical activity, lack of adequate sleep are the chief causative reasons for ever rising obesity cases across the globe (Zlobine 1861; Jung, 2014; Serin and Acar Tek 2019) [41, 16]. As a consequence of obesity, subsequently, in a long run it may link with several co-morbidities such as diabetes, hypertension, cardiovascular disorders or stroke it may also be due to genetic factors, high calorie intake and circadian disruption through regulating the metabolic genes, thereby optimizing nutrient uptake and digestion with respect to the food timings (Villanueva *et al*. 2019; Emily 2016) [34, 9]. Further, in recent decade the circadian timing system or circadian clock plays a crucial role in much biological process which is adapted to the light dark cycles due to earth rotation. Recent research, have unravelled the importance of such circadian adaptations long term/ short term disruptions are associated with many pathological impairment *viz.,* premature mortality, obesity, impaired glucose tolerance, psychiatric disorders, anxiety, depression and cancer progression. Hence circadian rhythm allows species to adapt their physiology to daily environmental variation tied to earth rotation. i.e. dark light hours, feeding schedule and sleep pattern. Any change in the pattern of these activities for long time results in disruption of circadian clock, whereas scheduling daily feeding under time-restricted feeding (TRF) without reducing calorie intake promotes gene expression and is known to reverse the effects of diet induced obesity (Valter D., 2016; Almaqashah *et al* 2022).

Drosophila, commonly known as fruit fly is widely used in studies as an experimental model as it shares high sequence homology with human genome. 75% of disease causing genes in human has homologs in *Drosophila* genome (Mirzoyan *et al*. 2019). Although, *Drosophila* is a well-known model for circadian studies, little is known about its circadian control of metabolism. Recent high fidelity animal studies better mimic human shift workers by using a protocol which maintains rats on a stable LD cycle but forces the rats to be active during the light (rest phase) and made to run on a wheel during work hours but free access to food and water. This work during the day is sufficient to cause a shift in some circadian rhythms, including activity and feeding, resulting in metabolic disruption, loss of glucose rhythamicity, inverted triglyceride rhythm and increase body weight (Salgado-Delagdo and Angeles-Castellanos and Buijs *et al* 2008; Emily *et al* 2016; Almaqashah *et al* 2022) [9, 26]. The other circadian oscillators such as those in liver are sensitive to feeding activity and regulate the expression of a large number of metabolic and physiological genes (Vollmers *et al*., 2009) [35]. Changing the feeding patterns brings about a change in levels of metabolites which play crucial role in daily activities of the organism. Although daily rhythmicity in the levels of numerous metabolites has been discovered, understanding of these metabolites is limited. Many attempts have been made to understand these aspects in model organisms, *Drosophila* emerged as model to study metabolic disorders because of the following reasons. Many metabolic pathway, physiological conditions, growth of *Drosophila* basically

share homologous pathway with mammals. Insulin like proteins, are released in response to high levels of circulating sugar and glucogon like molecule, adipokinetic hormone (AKH) is released in response to low levels of circulating sugar which is similar to humans (Colombani *et al* 2003; k*et al* 2010; Kim and Rulifson 2004; Lee and Park 2004) [7, 19]. Simple sugars from food are taken up passively from the digestive tract directly into the fat body where they are converted to trehalose, a nonreducing sugar (Nation, 2002; Ugrankar *et al* 2015; Wyatt and Kale, 1957) [21, 31]. Further fat body acts as afunctional homolog of both the liver and adipocytes in insects suggesting a consolidation of physiological function (Arrese and Soulages 2010) [2]. *Droosphila* insulin receptor homolog (DInR or InR receptor prove to be function similarly as mammalian insulin receptor and Insulin and insulin like signalling (IIS) pathway are shared between flies and humans (Fernadez-Almonacid & Rosen 1987; Das Dobens, 2015; Kannan and Fridell, 2013; Oldham 2011; Taguchi and White 2008; Teleman 2010) [10, 18, 22, 29, 30]. Fast feeding regulation of glucose, Glut1, accumulation of lipids in fat body and midgut in a dose dependent way mimic the major risk of T2D and related co morbid situations shares common physiology with mammals (Park *et al* 2014; Bodmer and Venkatesh 1998) [23, 4]. Hence in the present study, attempts have made to examine the temporal organization of fly feeding behaviour, Morphometry, wet body and dry body weight, Lipid estimation and longevity of the groups treated with High fat diet (HFD), High fat with Time restriction feeding (HFD+TRF) compared with the Normal diet with TRF and evaluated the effect of time restriction feeding on diet induced obesity. The results of the study demonstrated that feeding is under circadian regulation, and that a peripheral clock, at least in part, is involved in modulating feeding rhythms.

Materials and Methods

The material used in the experiments were of sigma unless mentioned specifically.

1. Flies/Rearing

Fly culture and diet preparation The *Drosophila melanogaster* Canton-s strain was obtained from *Drosophila* stock center, DOS in Zoology, University of Mysore**.** All flies used in the experiment were wild-type, Oregon R-Upon enclosing, flies were collected and housed on high fat media at approximately equal densities of 30 flies/vial until five generations. Females were then synchronously mated with males of the same genotype in a 2:1 ratio for two days. After mating, males were removed, and the females were divided equally into three groups and placed on one of the four corresponding media types (ND, ND+TRF, HFD, HFD+TRF). The fly groups were kept on their respective media for seven days, being transferred to new media every three to four days. Flies were kept in an incubator at 25°C on a 12L/12D light/dark cycle with consistent humidity.

2. Experimental setup

The strain of *Drosophila melanogaster* used in the present study is laboratory stock of wild-type, Oregon R. The experimental stocks were established initially on the corn wheat media and the four groups of flies aged for five days were put into four types of diet regimes.

Table 1

Group	Type of diet
I	Normal balanced diet (ND)
II	Normal diet with time restricted feeding (ND+TRF)
III	High fat diet (HFD)
IV	High fat diet with time restricted feeding (HD+TRF)

The high fat diet was prepared with 5% lard + 1% emulsifies (tween) and in time restricted feeding (TRF) the flies are fed only during 12 hours in a day. All four groups of flies were kept in incubator with a 12h dark /12h light cycle, 75% humidity, and a temperature of 25 °C. In the time-restricted period, all the adult flies were transferred to the *Drosophila* rearing chamber (population chamber) (n = 30) and reared for five generations on the same diet regime the food cups were introduced into the chamber only during the light hours 7am -7pm). For media preparation of 4 groups we followed the method of Almaqashah *et al* 2022.

3. Media Preparation (Normal)

The normal media was prepared by the following steps; Weigh corn flour, Jaggery, Agar and yeast powder separately and keep them aside. Take the 1180ml of water in a pressure cooker. Warm the water (approximate temperature 35ºC) and add corn flour. Mix thoroughly to remove any clumps. Add other ingredients (Jaggery, agar and yeast powder) and mix again. Allow to boil. Open the pressure cooker and mix thoroughly. Turn off the stove. Cool the media to 50-55 ºC (make sure that it is checked with thermometer). Add benzoate solution which has been mixed with ethanol, followed by propionic acid and mix thoroughly.

Composition

Table 2

Gajju's special media	Composition (for 1L)
Corn flour	24g
Jaggery	35g
Agar	9g
Yeast powder	15g
Propionic acid	4.4ml
methylparahydroxy benzoate	1.25g
Ethanol	25ml

4. Media Preparation (HFD)

The normal media was prepared by the following steps; Weigh corn flour, Jaggery, Agar and yeast powder separately and keep them aside. Take the 1180ml of water in a pressure cooker. Warm the water (approximate temperature 35ºC) and add corn flour. Mix thoroughly to remove any clumps. Add other ingredients (Jaggery, agar and yeast powder) and mix again. Allow to boil. Open the pressure cooker and mix thoroughly. Add lard to the media and mix well. Turn off the stove. Cool the media to 50-55 ºC (make sure that it is checked with thermometer) Add benzoate solution which has been mixed with ethanol, followed by propionic acid and mix thoroughly.

Composition of the Media

Table 3

Gajju's special media	Composition (for 1L)
Corn flour	24g
Jaggery	35g
Agar	9g
Yeast powder	15g
Propionic acid	4.4ml
methylparahydroxy benzoate	1.25g
Ethanol	25ml
Lard	50g

5. Food Intake Assessment

a. Quantification of Food intake in Larvae using dye method

Second instar larvae (10 No.) obtained from normal and test media were used to study feeding behaviour. Each larva was placed in a modified Delcour media cup containing normal and treated media with 2.5% (w/v) blue food dye (FD & C Blue Dye no. 1). The larvae were starved for an hour and later allowed to feed on the dye treated normal and test media for 15 minutes. Then they were transferred to Eppendorf tubes and frozen. These frozen larvae were homogenized by adding 200 µl of distilled water and made the volume to 1000 µl by adding 800 µl dist. H2O. We measured the OD at 629 nm using Spectrophotometer. The amount of food taken was measured from the standard graph made from serial dilution of the blue dye (Shell, B. C *et al* 2018) [27].

b. Combined proboscis-extension and blue dye assay

Groups of larvae were transferred onto fresh food medium as indicated containing 2.5% (w/v) blue food dye (F D & C Blue Dye no. 1). Vials were scored approximately every 2 minutes for proboscis extension and after a total of 30 minutes were transferred to Eppendorf tubes and snap frozen in liquid nitrogen (Wong, R. *et al* 2009) [38].

c. Colour Spectrophotometry

Larvae were homogenized in 200 mL of distilled water. A further 800 mL of distilled water was added and the suspension passed through a 0.22 mm Millex filter (Millipore Corporation, Bedford) to remove debris and lipids. The absorbance of the liquid sample was then measured at 629 nm [Hitachi U-2001 Spectrophotometer (Lambda Advance Technology Ltd., UK)]. Larvae were exposed to non-dyed food were used as the baseline during spectrophotometry. The amount of labelled food in the larvae was calculated from a standard curve made by serial dilution in water of a sample of blue food. Several methods for the indirect determination of food intake are introduced. One option for food intake monitoring is the use of trackable supplements such as radioisotope-labeled metabolites or nonabsorbable dyes (F D & C Blue Dye no. 1) In the case of dyes, flies are fed on the labelled, medium for a defined period of time. Subsequently, the amount of dye that has accumulated in the digestive tract, which reflects food intake, is quantified in fly lysates by using a spectrophotometer or fluorescence reader

6. Wet body weight measurement

For the determination of body weight, flies have to be sex-specifically pooled after pre feeding. Following transfer to sealable vials, groups of at least 10–20 animals are weighed with a precision scale, and the average body weight per fly is calculated (Staats *et al* 2018) [28]. To quantify the dry mass, the fruit flies are dried in an oven (e.g., at 60 ° for 24 h) before their body mass is determined with a precision scale by following the method of Burggren *et al* 2017**.** Calculate the difference between wet and dry weight, to calculate the weight we followed the method of Heynen, 2014, Five adult males were taken into a 1.5 mL Eppendorf tube as one replicate, and 3-5 replicates were measured for each group of flies using the Sartorius Mikrowaage MC5 (Satorius AG, Göttingen, Germany). The weight of each tube (empty) with flies was also determined by averaging the values of 3 replicates. Statistical analysis was done with Excel 2011 (Student's t-test). Each experiment was repeated at least twice for consistency (Heynen, 2014) [13].

7. Body Size assessment

Labelled a microscope slide with the id of each sample to be measured. Placed a small (~5 µl) drop of superglue onto the microscope slide using a 20 µl pipette tip. Mounted flies in the superglue. The microscope was set to the dissection to 5× magnification. Adjusted the microscope focus depth in each instance, so that the tip of the scutellum and the dorsal bristles are visible and in sharp focus. Loaded the 1 mm graticule image and draw a straight line across the length of the graticule and loaded desired thorax image. Drawn a straight line from the most posterior tip of the scutellum to the anterior edge of the thorax, angling the measurement line through the point where the dorsal bristles at the anterior edge of the thorax stop and captured the image.

In order to measure the body size, a microscope slide was labelled with the id of each sample to be measured. The flies were mounted on the slide by putting a small (~5 µl) drop of superglue onto the microscope slide using a 20 µl pipette tip. The microscope was set to 5× magnification. The microscope focus depth is adjusted in each instance so that the tip of the scutellum and the dorsal bristles are visible and in sharp focus. The 1 mm graticule image is loaded and a straight line is drawn across the length of the graticule. The desired thorax image is loaded and a straight line is drawn from the most posterior tip of the scutellum to the anterior edge of the thorax. The measurement line is drawn through the point where the dorsal bristles at the anterior edge of the thorax ends and image is captured (De Jong, G., & Bochdanovits, Z. 2003) [8].

8. Adult Dry Weight and Lipid Assay

The freeze-killed flies from preadult were sorted under the binocular microscope. Males were distributed in groups of 10 into clean dry vials, dried at 70°C for 36 hours, and weighed to the nearest micrograms. Lipid content was estimated by defatting the flies, tubes containing 1.3 mL ether. Lipid was extracted over a 36-hour period at room temperature of 25 ± 2°C with gentle agitation on a gel-rocker set to 2000 rpm. Ether was changed every 12 hours. At the end of 36 hours, flies were removed from the ether, washed with 1 mL fresh ether, dried at 70°C for 2 hours, and weighed to obtain lipid-free dry weights. The difference between dry weight "before" and "after" ether extraction was taken as the total lipid content. Five vials were set up per treatment per population (Asher, 2015) [3].

9. Longevity Assay

The longevity of reproducing flies was assayed. One day old flies were sorted in to vials containing approximately 4 ml standard media as four males + four females/ vial twenty such vials are maintained for each treatment group such as ND, ND=TRF, HFD and HFD+TRF totally 640 males and

640 females were assayed for longevity. Dead flies were scored and removed from the vials. Live flies were kept transferred until their death (Kamyshev, N. G. 1980) [17]

10. Climbing Assay

Twenty flies of each group (HFD, HFD+TRF, ND, ND+TRF population) were placed at the bottom of a 4″-long glass vial over which another 4″-long vial was inverted Glass vials were separated from each other after 30 seconds, and the number of flies in the top vial was counted for all the groups separately. Four climbing trials (akin to sampling with replacement) were made per vial. The climbing index was expressed as percentage of the number of flies that climbed to the top vial relative to the total number of flies tested (Gargano *et al*., 2005) [11]

11. Cafe Assay

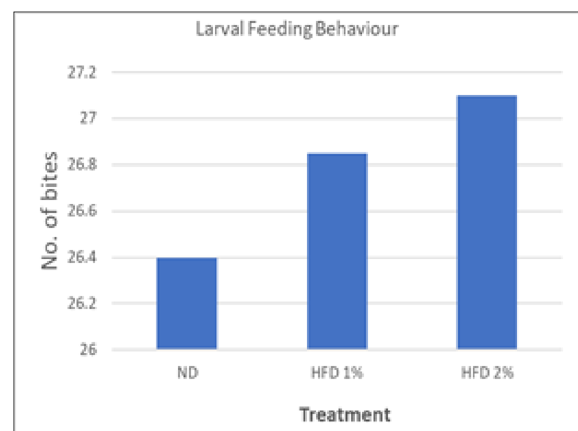
To quantify adult fly food intake, a capillary feeding (CAFE) assay performed according to (Ja *et al*., 2007) [14] with slight modifications. Prepared two chambers the inner chamber, had the flies, with a 1.5-cm diameter plastic vial to 2-cm length, Calibrated glass micropipettes filled with a liquid medium by capillary action were inserted through the cap, truncated 200uL pipette tips. The long-term experiment was conducted under a 12-h-light/12-h-dark cycle in a room kept at 25°C and70% humidity. The choice experiments were performed with two labeled capillaries, each containing a different food. Each experiment included an identical CAFE chamber without flies to determine evaporative losses (typically10% of ingested volumes), which were subtracted from experimental readings. Average values are given. All flies tested were1-week-old of the treated with HFD and untreated strain and transferred to the CAFE from this food. Except where otherwise specified, the liquid food used in the CAFE was sucrose 5% (w/v) All flies were habituated in the CAFE for 24 h, with medium, before the measurements were started.

Statistical Analyses

In all cases except lifespan analysis, the population means were used as the units of analysis. The significance of the difference between means was assessed using one-way analysis of variance. The difference among treatments was compared by Tukey–Kramer Minimum Significant Difference (MSDa0.05) Test. The significance of the difference between adult survival curves was analyzed using Kaplan–Meier log-rank test.

Results and Discussion

Obesity is a multifactorial disorder driven by a high-calorie diet, a hereditary predisposition, circadian disruption, etc. To assess the effectiveness of innovative lifestyle therapies and treat these conditions, there is an urgent need to simulate obesity and metabolic diseases in lab animals. This study achieves these goals using a *Drosophila* model of obesity. In the present the *Drosophila* model was established understand the impact of high fat diet induced obesity and the role of time restricted feeding in controlling the HFD complication. Our study in this regard has revealed time-restriction feeding and its benefits. The experiment aims to investigate the adverse effects of a high-fat diet on odd-time eating and high-fat diet with that of TRF. To address this problem, we choose *Drosophila melanogaster* flies that were fed with different food regime as high-fat diet (HFD), a normal diet (ND) as a control, HFD+TRF and ND+TRF. The biochemical assays were used to detect obesity, body weight, body size, longevity, and TRF normalizes the triglyceride level to reduce obesity in the HFD group was observed and TRF was found to have an effective response to obesity by reducing body adiposity caused by a high-fat diet. HFD-induced obesity is associated with an increased risk for diseases, including cancer, diabetes, and heart disease (Yuqing She., 2019). The polygenic nature of HFD-induced obesity makes it difficult to determine the relative contribution to each of these diseases. In order to examine these complicated interactions in a simple system, we established a HFD-induced obesity model in *Drosophila* to elucidate the underlying mechanisms. Quantification of Food intake in Larvae and adult flies was conducted using FD and C blue no. 1 dye assay. The larvae and adult flied were feed with blue food dye to study feeding behaviour (Figure. 1. A). In results, it was found that the number of bites were significantly increased from 26.4 to 27.1 in normal to high fat diet respectively. The no. of bites increased as the fat percent, this indicate the stability of this model to assess the further experiment of this study. The results were further confirmed in feeding assessment in adults using colorimeter assay. The colour intensity was increased with increase in fat percent in the fly (Figure.2. B). *Drosophila* fat bodies are considered equivalent to vertebrate adipose tissue, both metabolically and in their endocrine role. These results were also supported by similar studies indicating the efficiency of TRF diet (Villanueva, 2019) [34].



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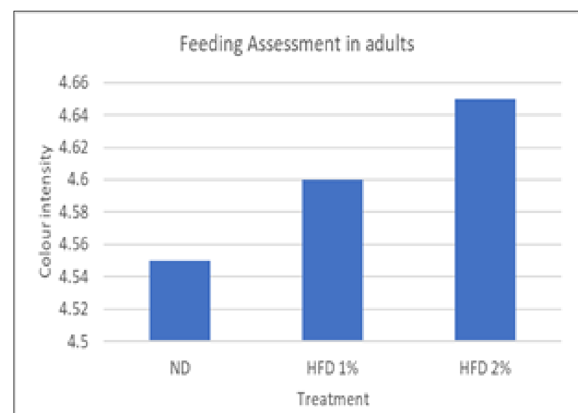

B

Fig 1: **a. Larval feeding Assay:** Larvae were allowed to feed the media which was normal diet (ND), High fat diet (HFD). Feeding counts were recorded under stereozoom microscope. b. Adult feeding Assay: Adults were allowed to feed the media with added colour in normal diet (ND), High fat diet (HFD). Feeding counts were recorded under stereozoom microscope. Data has been presented as Mean ±SEM.

To investigate the obesity among normal and high fat diet flies, fresh as well as dry body weight were calculated after 10th, 20th and 30th day of feeding (Figure. 2). Approximately 40 µg increase in weight of female *Drosophila* on 10th day, the body weight was found to be maximum on 20th day while decreased on 30th day of experiment. It was observed that flies received HFD-TRF were in control but there was no significant weight loss on 30th day in the female *Drosophila* (Figure. 2. A). However significant weight loss was observed in male flies. In dry body weight assessment a remarkable downfall was recorded in male as well as female flies (Figure. 2. B).

Lower body weight of fly body showed that diet induced obesity resulted in a greater proportion of lipid droplets with aberrant increase in weigh while, TRF reduced their body weight to 15% in10 days.

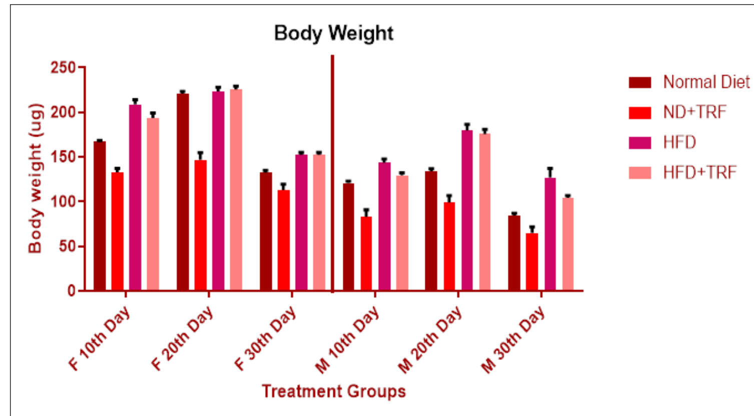

Fig 2: A. Fresh Body weight. Body weight was measured after treating with high fat diet (HFD) and Time restricted feeding (TRF) in comparison with flies fed with normal diet (ND). There is a significant increase in body weight after HFD and recovered after TRF. Data has represented in Mean ±SEM.

Further, the effect of high fat diet on body shape was examined. In the experiment, comparatively high body size was observed in HFD which was found to be lowered in HFD-TRF group of flies. Lower abdomen sections of fly fat bodies showed that TRF resulted in a greater proportion of lipid droplets with aberrant shapes and increased size (Figure. 3). TRF decreased the number of abnormally shaped fat droplets, as well as their average size, by nearly 2mm in both male and female flies. TRF also mitigated lipid deregulation associated obesity in flies, these results were also in agreement with some previous studies.

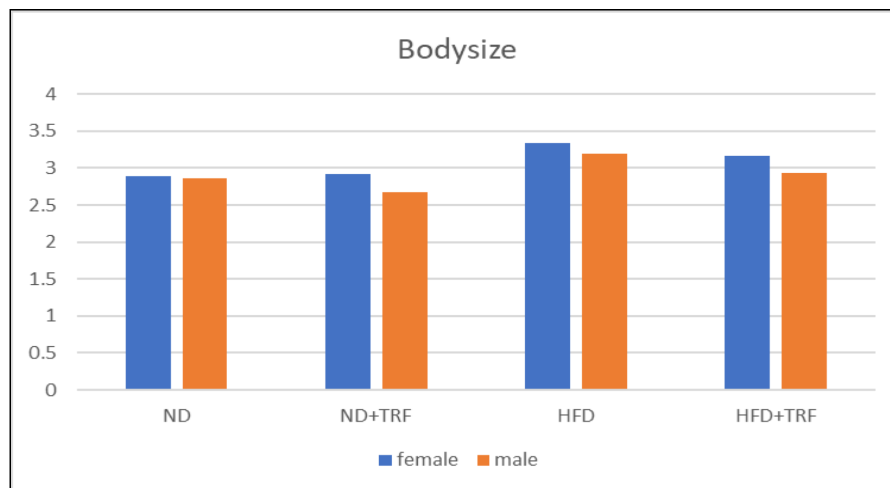

Fig 3: Body Size Assessment. Body size was measured after treating with high fat diet (HFD) and Time restricted feeding (TRF) in comparison with flies fed with normal diet (ND). There is a significant increase in body size in male and female flies with HFD and recovered after TRF. Data has represented in Mean ±SEM.

The geotaxis experiment was performed to identify a locomotion deficit associated with obesity and the effect of TRF on locomotion (Figure. 5). Human pathological obesity is frequently accompanied with impaired muscle function and insulin resistance in the muscles. In this work, we discovered that HFD gradually decreased *Drosophila* flying index and geotaxis activities, which provide a physiological readout of muscle performance. This impairment is linked to an increase in intramyocellular lipids IMCL deposition in muscle. Regular IMCL build up and depletion during high physical activity is also observed in endurance trained athletes, suggesting that IMCL accumulation alone may not affect muscle function.

(Villanueva *et al* 2019) [34] However, the progressive and tonic build up of IMCL may eventually affect muscle ultrastructure, which correlates with reduced flying index and geotaxis movement. Diet-induced obesity resulted in a higher proportion of lipid droplets with abnormal forms and increased size, according to lower abdomen sections of fly fat bodies. TRF reduced the quantity of improperly shaped

droplets as well as their average size in both male and female flies by about 20%.

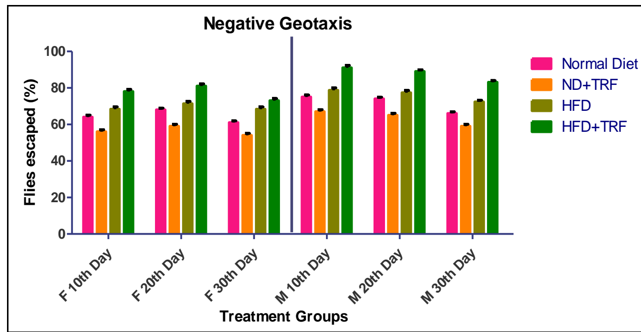

Fig 4: Negative Geotaxis. Negative Geotaxis was measured after treating with high fat diet (HFD) and Time restricted feeding (TRF) in comparison with flies fed with normal diet (ND). There is a significant changes in locomotory activity after HFD and recovered after TRF. Data has represented in Mean ±SEM.

We tested the effect of TRF on survival probability of *Drosophila melanogaster* using longevity assay. In agreement with age-dependent functional declines, both males and females exhibited an age-associated decline in flight performance and climbing ability (Figure. 6). Feeding the same flies, a HFD accelerated decreases in functional performance and survival probability. Comparison of age dependent survival curves it was found that flies in HFD-TRF group has the highest survival rate and longer survival probability. The survival probability was declined after 90 days as compared to normal diet with TRF which was found to be at 57th days in males. This trend continued throughout the experiment. TRF attenuated age-associated declines at most time points when animals were fed with HFD, and partially rescued performance in HFD flies. TRF benefits were found for both male and female flies. Similar observation was also detected in other studies (Victoria Acosta., 2022) [33].

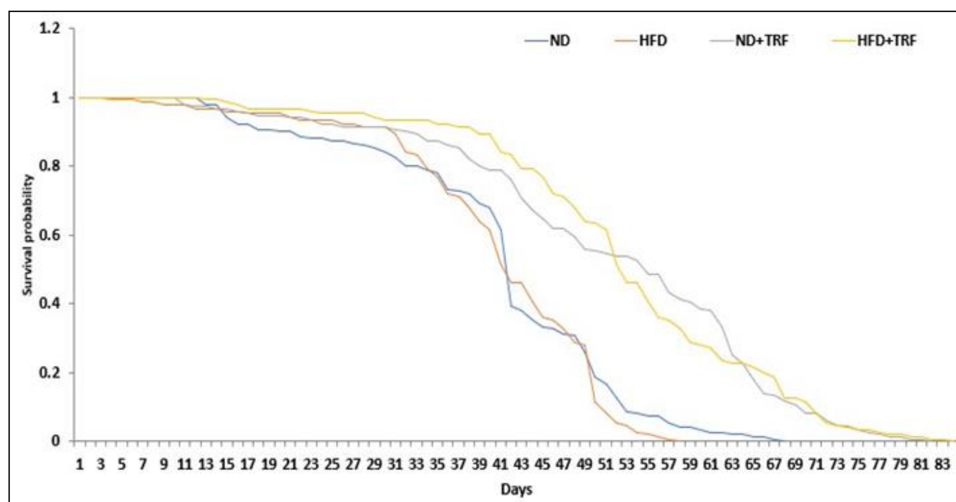

Fig 5: Kaplan-Meier survival probability curves High fat diet and time restricted feeding in *Drosophila melanogaster*

With devastating effects on human health, obesity, metabolic syndrome, and related diseases are at an all-time high (Chaix 2019) [6]. Utilizing model species, such as *Drosophila*, to quickly generate obesity and screen for genetic variables or natural and synthetic chemical substances to better understand metabolic imbalance is now possible thanks to the combination of the HFD regimen with the high throughput TAG assay. This could ultimately make a significant contribution to the development of new therapeutic approaches or metabolic illness solutions.

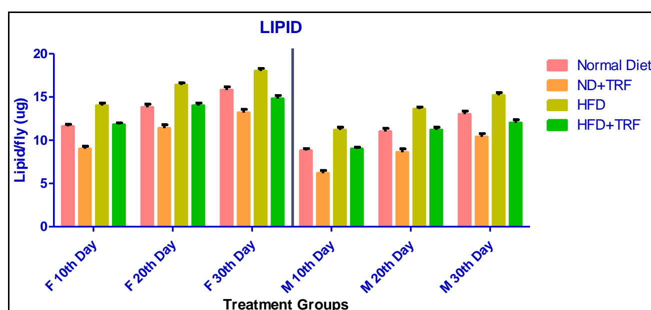

Fig 6: Lipid Content. Lipid content was measured after treating with high fat diet (HFD) and Time restricted feeding (TRF) in comparison with flies fed with normal diet (ND). There is a significant changes in lipid level after HFD and recovered after TRF. Data has represented in Mean ±SEM.

In *D. melanogaster*, as is the case with other species, there is sexual dimorphism between males and females. It is well known that females are larger, with more fat in their abdomens, than males. To test the effectiveness of time restricted feeding, TAG assays was performed to determine the differences in TAG content between males and females of standard laboratory wildtype flies. The data show that females have more whole body fat than their male counterparts (Figure 7). The data also showed that the assay is stable, with no variation in TAG quantification over time. These results are also supported by similar studies indicated the efficacy of TRF in obesity reversion. We found that consumption of HFD in *Drosophila* causes increased fat content that progressively accumulates over time. Another important finding is that after only 10 days of HFD feeding, we were able to induce a significant increase of fat content in these flies. 2-week old flies on a normal diet were collected, grouped by sex (males and females), weighed and ground up (5 flies per well) for TAG analysis. TAG assays were performed following the procedures described in this paper. The absorbance of each sample at 550 nm was read at different time-points (10th day, 20th day, 30th day) to determine eventual fluctuations in TAG quantification over time, fat content variation in different population sizes (3 and 5 flies) of flies, and differences between male and female TAG contents. The results showed that females

accumulate more fat than their male counterparts, TAG measurements do not fluctuate up to 30 days after the reaction incubation at 37 °C. Also, the mean TAG levels remain unchanged between TAG assays using 5 flies. The rapid induction of fat accumulation mediated by conserved cellular and molecular processes controlling lipid and glucose metabolism is advantageous for many obesity-related studies, such as diabetic or lipotoxic cardiomyopathy (Figure 8).

Capillary Feeder (CAFE), a technique that enables accurate, real-time assessment of intake by single fruit flies or groups of them on a time scale ranging from minutes to days. We performed the first quantitative analysis of *Drosophila melanogaster's* prandial behaviour using this method. Our findings enable the division of eating into discrete periods of intake, defining two distinct parameters—meal volume and frequency—that can be separated and are therefore probably independently regulated. Our long-term studies also reveal that over the course of 24 hours, flies can consume up to 1.7 times their body mass. The CAFE can also be used to track oral medicine administration in addition to appetite research. As an example, we tested the effects of dietary supplementation with two substances, paraquat and ethanol, on food intake and preference using the CAFE (William, 2007) [37]. Fly larvae in the CAFE eat liquid food from graded glass microcapillaries. The meniscus is plainly visible as it descends, making it possible to quantify consumption continuously and clearly.

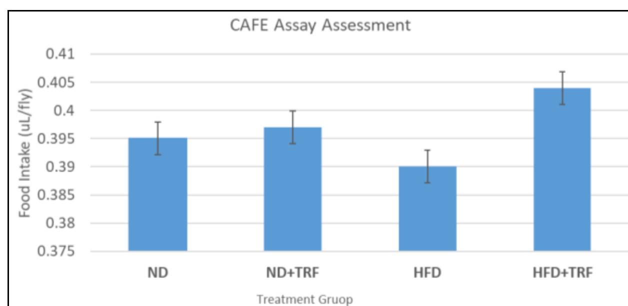

Fig 7: CAFE Assay. The total food intake was measured by capillary feeding (CAFE) assay. Data has represented in Mean ±SEM.

When the food intake was quantify after 24h of incubation, it was found that ND group consumed 0.395µl ±0.01, ND+TRF consumed 0.397µl ±0.005, HFD consumed 0.39µl ±0.005 and HFD+TRF consumed 0.404µl ±0.04 respectively. Food consumption in flies with HFD and time restriction feeding has the highest consumption which indicate the improvement in metabolic activity of flies and as compared to the flies consuming high fat diet. These results confirm the reverse efficacy of TRF in obesity by improving the metabolic efficiency hence food consumption was also improved which ultimately help in reducing the lipid concentration and weight loss.

Conclusion

High fat diet induced obesity is associated with an increased risk for diseases, including cancer, diabetes, and heart disease. In the present study, the impact of HFD and efficacy of time restricted feeding in obesity was examined in *Drosophila* Species. The flies which were induced with HFD were found to have more body weight and body size as compared to flies received normal diet. However there was a significant control in body weight and size of flies which were simultaneously feeded with time restriction feeding. As the lipid accumulation was also remarkably lowered in HDF-TRF group the muscle activity was also found to be increased in male as well as female flies. The survival probability was also found to be reduced in HFD-TRF. Conclusively, our findings suggest that TRF regimen might be a potential novel non-pharmacological strategy to reverse the high fat diet induced obesity.

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