Effect of Quercetin on Growth and Mortality of High Fat-fed Drosophila Melanogaster

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Abstract: Association of obesity with high fat diet is no longer debatable. Obesity causes hyperlipidemia and upregulates the generation of several metabolic by-products such as reactive oxygen species (ROS). Excess generation of ROS is toxic to the cell that often induces DNA damage and eventually induces cell death. Accumulation of lipid along with high level of ROS increase mortality and impair organisms' growth. Herein, we have used *Drosophila melanogaster* as a model organism to assess the accumulation of lipid by commonly used dietary fat (commonly known as *ghee*) in Asia and its effect on the growth and mortality. Synthetic polyphenol, quercetin was used along with the high fat diet to address if this particular polyphenol can be used as a protective health supplement to minimize lipid-related toxicity. Our study suggests that high fat diet impairs the growth of *D. melanogaster* as detected by measuring total protein, whereas this is not affected by quercetin. It also appears that quercetin alone can induce high level of lipid accumulation and this is further enhanced in presence of dietary high fat (*ghee*) in these experimental flies.

1 INTRODUCTION

Drosophila melanogaster, commonly known as fruit fly, is one of the widely used experimental model in life sciences. For decades, this dipteran insect is being used to investigate the genetic basis of inheritance and hereditary disorders due to the simplicity in the genome structure (Sobels and Vogel, 1976, Adams et al., 2000, Palu et al., 2017). In recent years, *D. melanogaster* has shown to be a useful model organism in studying human disorders such as Diabetes, Alzheimer's, Parkinson and most recently, neuro-associated disease (Zhu et al., 2014, Lau et al., 2015, Vanhauwaert and Verstreken, 2015, Zhao et al., 2015, Prussing et al., 2013, Lenz et al., 2013). Most biological architectures and mechanisms found in the fruit fly which affects the development and lifespan of the organism, are nearly similar to those in human. The fly offers many advantages as an investigative biological tool due to its rapid rate of proliferation and relatively inexpensive and easy to culture in laboratories.

Several research findings suggest that *D. melanogaster* can be used as a model for obesity or obesity related disorders (Ruden et al., 2005, Rovenko et al., 2015, Pospisilik et al., 2010, Padmanabha and Baker, 2014). The rising incidence of obesity is believed to be due to consumption of high fat and high carbohydrate containing diets. Though several other factors are responsible for this condition, health researchers mainly pointing to the increased uptake of fat and carbohydrates. Obesity often leads to the more complicated diseases which are the consequences of metabolic imbalance and increased oxidative stress. Hence, polyphenolic compounds are extensively used in health supplements that mainly act as anti-oxidants and prevent the oxidative stress-induce damages in the body.

In this experiment, we have used *D. melanogaster* to assess the effect of commonly used dietary animal fat (ghee) along with a synthetic polyphenol (quercetin) on their growth, ability to metabolize lipid, and mortality.

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2 MATERIAL AND METHODS

2.1 Establishing *Drosophila melanogaster* culture

Wild-type *Drosophila melanogaster* were captured from the local fruit market in Brunei. The flies were cultured in a clean, cylindrical, plastic container at room temperature. The entire experiment was conducted at the University of Brunei Darussalam.

2.2 Preparation of *Drosophila's* Standard Diet

We have used our formulated diet that we have optimized at the beginning of the experiment. The diet contained 100g of banana (Hotil Banana), 25g of rice flour, 4g of agar and 1g of bakery yeast (Mauri-pan) as a source of protein. After blending these ingredients in 100ml of water, the mixture was boiled briefly and poured into the feeding containers. In order to observe the effect of different diets to the growth and mortality of *D. melanogaster*, 7 % animal fat (v/ v, Ghee, Q. B. B) and 0.1% quercetin were added.

2.3 Experimental Flies Separation and Larval Collection for Protein and Lipid Assays

Parental flies (F-generation) were separated into a new container once the F_1 generation pupae had emerged in the experimental box. New food was introduced to the new container upon separation of flies. The number of dead F flies in the container was monitored regularly for 14 days to check the mortality rate the parental flies. During this observation, all the flies were fed with fresh food every 3 days to avoid the mixing of F1 flies with the parental flies. F1 flies were collected in batches of same age and used for protein extraction. The third-instar larvae (final stage before pupal stage) were obtained from the containers containing the F1 adult generation. 20 larvae from each experimental unit were isolated and used for biochemical analysis.

2.4 Protein Extraction and Quantification

Protein Extraction from Adult Flies: The preserved flies in the Eppendorf tubes were homogenised in 300µl of RIPA buffer mixed with protease inhibitor by using sterile plastic pestles while keeping the homogenate on ice. The homogenized samples were incubated on ice for 20 minutes for complete lysis of the cell membrane. Precipitation of grinded sample was minimised by vortex. The homogenate was centrifuged at 12, 000 rpm for 15 min at 4ºC and the supernatants containing cytosolic protein were transferred in a new tube. 250 µl of supernatant containing cytoplasmic protein was transferred into a new tube and stored in -80ºC.

Protein Extraction from Larvae: Slightly different technique was implemented to extract protein from larval samples. Prior to the extraction, the larvae were washed in PBS at least twice and incubated for 5min. This step allowed the removal of any food residues that might present on the external surface during larval collection. The cells in larval samples were lysed by the same method in carried out in the adult samples. However, the lysed samples were centrifuged at the speed of 2, 000 rpm for 5 min in 4ºC.

Protein Quantification of Adult Flies and Larval Samples: Total protein content was quantified by Bradford Reagent (BioRad) according to the manufacturer's protocol. Bovine Serum Albumin (BSA) was used as standards and the absorbance of the samples and standards was recorded using a microplate reader at OD595nm. Standard curve derived from the BSA standard was used to quantify the protein samples.

Lipid Quantification: 20 µg of protein sample was loaded into a clean microfuge tube (Eppendorf tube) and was spun-down briefly to bring all the contents to the bottom of the tube. The sample was stained with 100 µl of Oil Red-O (ORO) dye (Sigma) that selectively binds to neutral lipids. The tube was inverted twice to ensure proper mixing of the dye with the protein samples and then centrifuged at maximum speed (12, 000rpm) for 10 min at room temperature (25ºC). The supernatant was discarded entirely and the pelleted samples were washed with distilled water to remove any residual unbound ORO dyes. Second centrifugation of sample was applied at maximum speed for another 10 min at room temperature. The supernatant was discarded and 120 µl of isopropanol was dispensed into the reaction. This allowed the bounded ORO to be released from neutral lipids. The sample was vortexed briefly followed by incubation at room temperature for 10 min. Sample was centrifuged again at maximum speed for 10 min. The supernatant containing the ORO dye was eluted out and transferred to a 96-microplate for quantitative analysis. In microplate, 50 µl of the supernatant from each sample was transferred to the well in duplicates while 50 µl of isopropanol was used as blank. Oil-red-

O absorbance was recorded at OD515nm using a microplate reader. Lipid content was presented upon normalisation with total protein content measured from Bradford assay.

3 RESULT

3.1 Effects of Different Diets on the Growth of *D. melanogaster*

In order to assess the growth of the experimental organisms in different growth media, we have cultured *Drosophila melanogaster* in high fat diet (7% ghee or 7% olive oil) with or without quercetin (0.1%) while normal diet-fed *D. melanogaster* were used as a control. The effects of quercetin was also investigated in high carbohydrate containing diet using 0.75M sucrose (Fig.1).

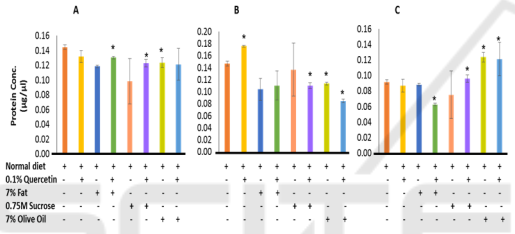

Figure 1: Protein contents per parental adult *Drosophila*, F generation, (A); F1 generation (B); and F2 larva (C) cultured in different diets (as indicated). *P<0.05

In F-, and F2-progenies, protein contents were not affected by quercetin compared to the control, but a significant increase was observed in F1 flies (Fig.1 B). High fat diet significantly reduced the amount of protein in F1 flies, suggesting a long term treatment with high fat diet might impair their growth. A similar trend was observed in sucrose + quercetin and olive oil containing diets (Fig. 1 B).

Interestingly, we have not observed any difference in terms of larval protein contents between quercetin and high fat diet with control series (Fig.1 C). However, larval growth was significantly affected in the quercetin containing high fat diet ($P<0.05$). In order to avoid any residual effect of the normal diet on the parental fly stocks (F generation) in their early life cycles, we have used F2 larvae to confirm our findings on the effect of these media on their growth. These F2 larvae were derived from F1 flies, both progenies were exclusively reared in the experimental media as indicated in the figures. Sucrose + quercetin and olive oil containing diets significantly increased the amount of proteins in these larvae (Fig.1 C).

3.2 Amount of Neutral Lipids

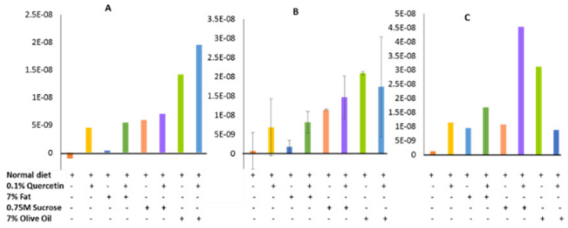

Figure 2: Absorbance (OD 515nm) of the Oil-red-O stained samples after normalizing with total protein. (A) F generation, (B) F1 generation. and (C) F2 larvae.

Next, we proceeded to estimate the fat contents in these model organisms. In f, f1, and f2 progenies, cytosolic lipid accumulation was not observed in normal diet feeding groups. F and f1 flies grown in high fat diet alone accumulated a marginal amount of lipids (fig. 2. A & b). Interestingly, high fat (animal fat, ghee) diet induced highest amount of lipid in these flies only in presence of quercetin, whereas quercetin alone induced remarkably high level of lipid. Both sucrose and olive oil induced significantly high level of lipids with or without quercetin (fig. 2 a & b).

The f2 larvae grown in different experimental growth media. In all the experimental media, accumulation of neutral lipids were significantly higher compared to the control diet. High fat diet, as expected, induced significantly high level of lipid accumulation compared to the control. As like f ad f1 flies, we have also observed that quercetin alone induced lipid accumulation similar to the high fat fed larvae and the amount of neutral lipid was highest when the larvae were grown in a combined diet of quercetin and high fat (fig. 2c). This could be due to the combined effect of quercetin and ghee as both of them are able to induce lipid accumulation independently as we have observed. In these larvae, sucrose + quercetin and olive oil containing diets induced significantly high level of neutral lipids, whereas quercetin + olive oil containing diet tends to reduce the lipid contents (fig. 2 c).

3.3 Mortality in Different Experimental Diets

The above initial findings led to observe the mortality of these flies grown in different media. High fat-fed *drosophila* had significantly higher mortality rate while the quercetin alone reduced their mortality in the parental stock (f and f1) (fig.3).

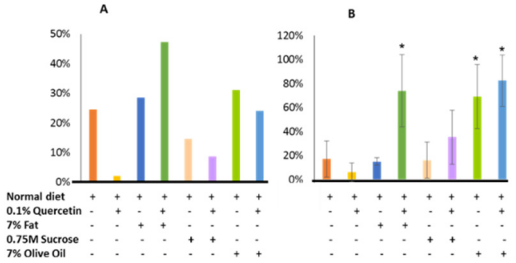

Figure 3: Percentage of mortality observed during the first two weeks of culture. (A) F generation, (B) F1 generation.

However, the trend was different when we have done similar study with F1 flies. In this experimental unit, it has been observed that quercetin induced higher mortality compared to the control, whereas high fat diet did not. Combined diet high fat and quercetin somehow induced significantly very high percentage of mortality (Fig.3. B). In all those experimental series, addition of sucrose did not increase their mortality significantly. Unsaturated fat, olive oil, induced high mortality rate as observed in animal fat (ghee) treated group.

4 DISCUSSION

Ghee, a commonly used animal fat, is produced from milk that contain a mixture of saturated and unsaturated milk fat. The effects of ghee on human body is still under debate with conflicting findings. As this is being used in several parts of Asia for traditional dishes, we were interested to investigate the effect of ghee in our experimental wild-type *Drosophila* model. Excess lipid accumulation often induces higher lipid metabolism that eventually causes lipid peroxidation and generate reactive oxygen species (ROS). Antioxidants are receiving increased attention due to their property to remove ROS from the body. Hence, we have used quercetin, a known antioxidant, to assess if this synthetic polyphenol is able to minimize the lipid-mediated oxidative stress in *Drosophila* reared with 7% ghee containing diet.

In this study, we have observed that cytosolic neutral lipid accumulation was remarkably high in quercetin treated flies and larvae. This might be due to the quercetin-mediated enhanced metabolism that allows these *D. melanogaster* to utilize dietary carbohydrate for *de novo* lipogenesis. The process might be further enhanced in presence of high level of dietary fat (ghee). Quercetin is known to possess beneficial effects on human and we have observed a similar phenomenon where it reduced mortality of the experimental models (*D. melanogaster*). This study raised the possibility that quercetin may also induce lipogenesis apart from its anti-oxidative property. The data presented here are based on our initial findings. In order understand the metabolic consequences, similar experiments need to be done using different experimental conditions such as varied concentrations of quercetin, carbohydrate, and animal fat.

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