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Influence of dietary factors on the longevity of *Drosophila*

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Abstract
Dietary restriction or modifying the protein carbohydrate ratio can influence the lifespan in *Drosophila melanogaster*. The effect of sugar and protein in the diet on the longevity of the fly was studied. Increased levels of dietary yeast resulted in higher levels of protein storage in the body. Adiposity attained by the fly was strongly correlated with the amount of sugar in the diet and inversely related to the concentration of dietary yeast. Longevity showed little relationship to the caloric content of the food, but a strong relationship between the carbohydrate protein ratio of the dietary source and lifespan was noted.

Keywords: *Drosophila*, yeast, sugar, longevity

1. Introduction
The lifespan of an organism is dependent on both extrinsic factors such as nutrition and intrinsic factor like the genetic makeup [1]. Dietary restriction extends lifespan in a range of species, and it delays age-dependent deteriorations in mammals [2]. Restricting nutrients without malnutrition definitely influence the lifespan and reduce age-dependent decline and diseases in virtually all species [3].
In fruit fly, *Drosophila melanogaster* lifespan responds to the macronutrients present in their diet: yeast as the source of protein and sucrose as the main carbohydrate. Lifespan is maximized at intermediate concentrations of each nutritional component [4, 5]. Flies fed on a high-sugar diet accumulate storage triacylglycerols (TAGs), becoming obese, develop insulin resistance and even heart disease [5-7]. However, the physiological responses to the two dietary components are different. Dietary components may act independently of their role in nutrition to modulate intracellular signaling pathways.
The choice of diet alone can greatly influence lifespan and can interact with genetic factors to produce diet-specific effects on longevity. Furthermore, some common food base alternatives like yeast extract instead of lyophilized whole brewer's yeast can dramatically shorten lifespan [8], leaving open the possibility that the food itself can cause stress to the organism that may impair the longevity. Adverse environments, may lead to the activation of stress-inducible factors like the expression of heat shock proteins that are known to influence adult longevity [9].

Methodology
Drosophila melanogaster were maintained at 25 °C, 60% humidity in a 12-hr dark/light cycle under conditions of controlled larval density in cornmeal-sugar-yeast 'larval' media for at least two generations prior to experimentation. Adult flies were collected within 24 hr of emergence and placed on 10 $g \cdot dL^{-1}$ sugar/yeast food for 72 hr to mate. Mated flies were then collected under light CO_2 anesthesia and females were placed into vials containing the experimental food type. Vials were changed every 2 or 3 days. For all experiments, flies were sampled by freezing ($^{-}$20 °C). Each nutrient regime contained in independent vials was used in triplicates with a density of 25 females. All samples were processed within 2 weeks of freezing time.

Metabolic assays
Adult females were sacrificed on 13th day after following 10 days of exposure to a specific dietary regime. For each replicate, five frozen females were homogenized in 300 µL PBS/0.05% Triton-X using a tissue processor. Samples were then filtered and used immediately for analysis. TAG assay was performed by adding 10 µL of fly homogenate to 200 µL of the Infinity Triglyceride Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and incubating at 37 °C for 15 min with constant agitation.

Concentrations were estimated based on absorbance values at λ520 nm and compared to glycerol standards.
Protein assay was performed according to the manufacturer's protocol using 5 µL of filtered fly homogenate combined with 200 µL of bicinchoninic acid reagent (Sigma- Aldrich, St. Louis, MO, USA) with bovine serum albumin as the standard.

Lifespan assays
A fixed number of eggs were washed with 70% ethanol and placed into bottles containing 30 mL of cornmeal-sugar-yeast 'larval' media. Resulting progeny were collected within 24 hr of eclosion and placed into bottles containing 15 mL of 10 g·dL^{-1} sugar-yeast media for 72 hr to allow mating. Female flies were collected under CO_2 anesthesia and placed into vials containing one of the 25 nutrient regimes at a density of 25 flies per vial. Vials were placed on their sides to allow access to both food and nonfood surfaces at advanced ages when flying is impaired. Deaths were recorded and flies transferred to fresh media every other day.

Experiment media
The experiments were designed to include five different concentrations each of sucrose and brewer's yeast (2.5 g·dL^{-1}, 5 g·dL^{-1}, 10 g·dL^{-1}, 20 g·dL^{-1} and 40 g·dL^{-1}) added to 100ml water, 1.5 gm agar and 0.3 ml Propionic acid.

Result
Flies were homogenized, and TAG was determined by spectrophotometric analysis. The level of adiposity attained by the adult fly was strongly promoted by the amount of sugar in the diet (Fig. 1). In contrast, the concentration of dietary yeast was inversely related to the amount of fat storage, suggesting that increased protein availability effectively suppressed fly adiposity. Yeast had a stronger suppressive effect on TAG storage in the presence of lower dietary sugar.

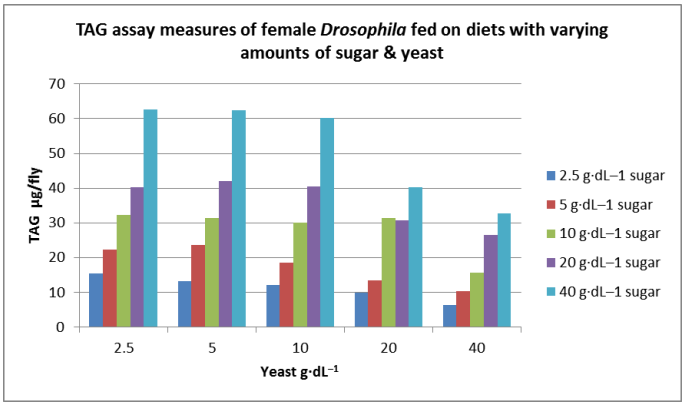

Fig 1: TAG assay measures of female *Drosophila* fed on diets with five different concentrations each of sucrose and brewer's yeast like 2.5 g·dL^{-1}, 5 g·dL^{-1}, 10 g·dL^{-1}, 20 g·dL^{-1} and 40 g·dL^{-1}.

The protein content in the body of flies was primarily determined by the concentration of yeast in the medium (Fig. 2). Increased dietary yeast resulted in higher levels of protein storage. Dietary carbohydrate had a much smaller effect, although it did promote a slight variation in steady state protein levels. The weight of the flies mirrored protein levels, with high levels of dietary yeast leading to heavier flies and increased carbohydrate availability leading to lighter flies.

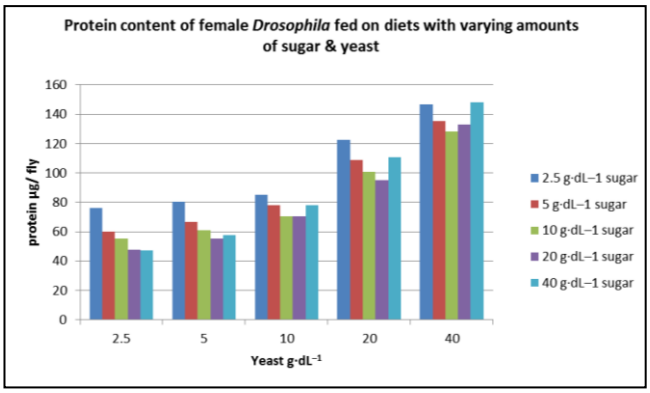

Fig 2: Protein content of female *Drosophila* fed on diets with five different concentrations each of sucrose and brewer's yeast like 2.5 g·dL^{-1}, 5 g·dL^{-1}, 10 g·dL^{-1}, 20 g·dL^{-1} and 40 g·dL^{-1}.

The data establish that diet composition acutely alters the body composition of flies. TAG levels were maximized in a low-protein/high-carbohydrate diet and minimized in a high-protein/low-carbohydrate diet. Yeast availability suppressed TAG storage and dietary sugar suppressed protein storage. Thus, the sugar/yeast ratio of the diet determined the amount of TAG and protein stored in the body of *Drosophila*.
To characterize the impact of each dietary component on fly longevity, we measured the lifespan of female flies that were maintained on each of the 25 different food treatments throughout their adult lives. Most dietary restriction experiments limit the animal's access to all nutrient components. Accordingly, we observed a maximum response to longevity study with a medium having 1:1 ratio of sugar and yeast (Fig. 3).

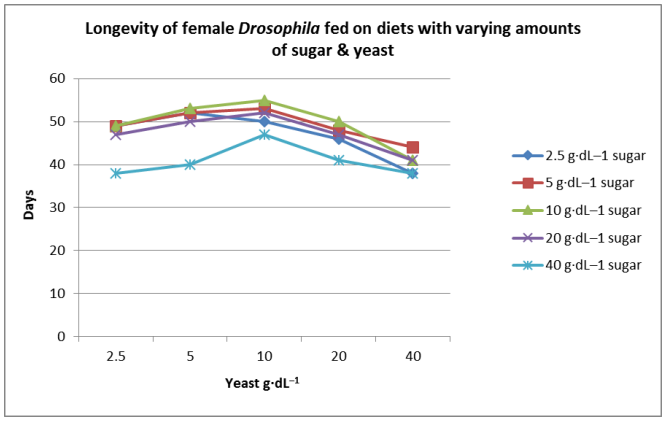

Fig 3: Longevity of female *Drosophila* fed on diets with five different concentrations each of sucrose and brewer's yeast like 2.5 g·dL^{-1}, 5 g·dL^{-1}, 10 g·dL^{-1}, 20 g·dL^{-1} and 40 g·dL^{-1}.

It was interesting to note that the adult body composition and longevity are not dependent on the caloric content of the food. The caloric content of brewer's yeast per unit mass, is nearly equivalent to sucrose [4]. Therefore, assuming that accessibility in the food is roughly equivalent for each nutrient, a 40 g·dL^{-1} yeast/5 g·dL^{-1} sucrose diet is calorically equivalent to a 5 g·dL^{-1} yeast/40 g·dL^{-1} sucrose diet. The longevity associated with these two diets, however, are significantly different as seen in Fig. 3.

Discussion

Flies exposed to a high-glycemic diet exhibited enhanced fat storage in their body. Fruit flies store energy from surplus calories in the form of TAG in specialized lipid-droplet-containing cells, and they release this energy for utilization via lipolysis as free fatty acids [10]. In our experiments, adult females were sacrificed at 13th day of adult age, following 10 days of exposure to a specific dietary regime. Although TAG assay measures all forms of glycerides, tri, di and mono, we assumed it accurately represented total TAG, considering that over 90% of fat body lipids are in the form of TAG in insects [11].

Dietary conditions have significant impact on age-associated physiological changes. Under most circumstances, the body composition of flies tend to remain relatively constant, with protein and fat levels holding steady or modestly decreasing throughout life [12].

Estimation of the absolute quantity of food uptake in *D. melanogaster* is difficult, and published results are conflicting [13, 14]. The complex effects of nutritional components on body composition and lifespan in flies provide a broad perspective for investigating the mechanisms of dietary restriction. Dietary restriction extends lifespan in species as diverse as yeast [15], nematode worms [16], and flies [17], and it is the most powerful modulator of the aging process known in mammals [18].

Conclusion

Organisms exposed to changing environment show variation in their biochemical reactions and morphology**.** Diet can have a significant impact on many aspects of fruit fly. Diet containing varying amounts of sugar and protein showed specific effects on the body composition and lifespan of *Drosophila*. The concentration of sugar in the diet influenced the adiposity of the fly, while the level of protein affected weight gain. The concentration of both sugar and protein in the diet greatly influenced the longevity of the fly.

Acknowledgement

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