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## RESEARCH ARTICLE

### DOES THE COMBINED GLUCOSE AND VITAMIN C NUTRITIONAL REGIMENS EXTENDS THE LIFE SPAN OF DROSOPHILA MELANOGASTER?

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#### ABSTRACT

**Back ground:** In most invertebrate systems, dietary restriction is applied somewhat non-traditionally in that food quality, rather than quantity, is manipulated through dilution of the nutritional components in the medium. To clarify how these nutritional factors influence longevity, nutrient consumption and lifespan, we were measured on a series of diets with varying glucose and vitamin C content. **Methods:** The fly stock is assessed for this experiment is *Drosophila melanogaster*. The stock is obtained from the *Drosophila* stock centre, Mysore, India. For the dietary restriction assays, different concentrations of Glucose (3g/100ml, 5g/100ml and 7g/100ml), vitamin C (0.25mg/100ml, 0.5mg/100ml and 0.75mg/100ml) and both Glucose plus vitamin are formulated in the diet. For longevity analyses, we used the modified protocol of Luckinbill and Clare, 1985. **Results:** The flies fed with combination of Glucose plus Vitamin C resulted in an increased life span followed by Vitamin C and Glucose in both males and females when compared to control. However LD of Glucose fed flies have resulted significantly decreased lifespan. Interestingly female flies have shown significantly increased life span in all the concentrations. **Discussion:** In several studies has been demonstrated that increased intake of protein may increase protein synthesis, decrease protein breakdown, reduce fat accumulation, and increase fat-free mass. The flies fed with combination of Glucose plus Vitamin C resulted in an increased life span followed by Vitamin C and Glucose in both males and females when compared to control.

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## INTRODUCTION

Dietary Restriction (DR) increases the life span in a variety of organisms such as yeasts, nematodes, fruit flies and mammals (Lin *et al.*, 2002; Partridge *et al.*, 2005; Walker *et al.*, 2005). It involves limiting dietary energy intake to improve health and retard ageing. In human subjects, caloric restriction has been shown to cause beneficial metabolic, hormonal and functional changes (Fontana and Klein, 2007). Dietary restriction, whereby food intake is reduced without leading to malnutrition, extends lifespan in many organisms: yeast (Kaerberlein *et al.*, 2007), invertebrates (Piper and Bartke 2008) and mammals (Weindruch and Walford 1988), including primates. In order to maximise its genetic contribution to posterity, an organism must appropriately direct the use of nutrients to traits such as growth, reproduction and repair (Richard *et al.*, 2009). Whether mechanisms of lifespan extension are conserved between humans and model organisms is unknown. The physiological mechanisms underlying lifespan extension by CR have yet to be identified.

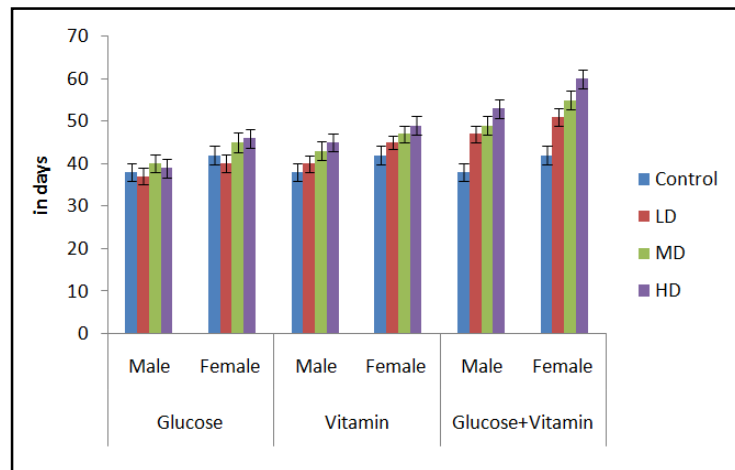
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*Drosophila melanogaster* is often used as a model organism in studies of physiological and evolutionary responses to various forms of stress (Hoffmann *et al.*, 2003; Sinclair *et al.*, 2007; Kristensen *et al.*, 2008a). However, there is little information about how nutrition affects life history traits (Watts *et al.*, 2006) and performance during stress, and the importance of diet is often underestimated in experimental designs (Prasad *et al.*, 2003). Hence, there is a growing need to investigate diet-related effects behind variation in traits of importance for fitness. Many organisms face a challenge of meeting their optimal nutritional requirements for somatic and reproductive growth under natural conditions (Raubenheimer and Simpson, 1999). During life, body tissues constantly require a specific quantity and proportion of nutrients, in order to attain optimal growth and performance (Bauerfeind and Fischer, 2005). Deficiency or imbalance of e.g. fat, carbohydrate or protein can affect characters such as somatic growth and reproduction. Adult *Drosophila* feed on soft rotting fruits that are rich in sucrose, fructose and glucose like. Apples, peaches, grapes and pears. In a classic series of experiments, Wigglesworth (Wigglesworth 1949) determined that these sugars provide energy for adult flight.

Table 1. Enriched food media administered to *D. melanogaster* flies

Diet→ Components↓	Control	Glucose			Vitamin C			Glucose plus Vitamin		
		LD	MD	HD	LD	MD	HD	LD	MD	HD
Water	1000ml	1000ml	1000ml	1000ml	1000ml	1000ml	1000ml	1000ml	1000ml	1000ml
Agar	10g	10g	10g	10g	10g	10g	10g	10g	10g	10g
Soji	100g	100g	100g	100g	100g	100g	100g	100g	100g	100g
Glucose	-----	30g	50g	70g	-----	-----	-----	30g	50g	70g
Vitamin C	-----	-----	-----	-----	25mg	50mg	75mg	25mg	50mg	75mg
Propionic acid	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml	7.5ml
Jaggery	100g	100g	100g	100g	100g	100g	100g	100g	100g	100g

Figure 1. Mean ( $\pm$ SE) Longevity of *Drosophila melanogaster* on exposure to Glucose, Vitamin C and Glucose plus Vitamin C

Flies that were depleted of muscle glycogen by flying them to exhaustion, resumed flight within two minutes when fed glucose, sucrose, fructose, mannose, maltose or trehalose. In contrast, some sugars such as arabinose were completely ineffective. A similar relative value of sugars was established by Hassett 1948, who counted the number of flies that survived when provided these sugars as the sole food source. Sucrose, glucose and fructose supported survival whereas arabinose and xylose were very poor. The aim of this study was to test if larval nutrition affects the ability of adult flies to cope with a number of different nutritional stress challenges, including Glucose, Vitamin and Vitamin+Glucose. As Vitamin and carbohydrates are the most important macronutrients for *Drosophila* larvae in natural food sources (Mattson, 1980), we choose to focus on these two very different dietary regimens. In order to test how these growth media affected life span of *Drosophila melanogaster*.

## MATERIALS AND METHODS

***Drosophila melanogaster* strain and culture condition:** The fly stock was assessed experiment was *Drosophila melanogaster*. The stock was obtained from the *Drosophila* stock centre, Mysore, India. The fly stock was cultured in standard wheat cream agar medium containing wheat flavour, jaggery, agar, yeast and propionic acid. Flies were cultured under uncrowded culture condition at  $22\pm 1^\circ\text{C}$  (rearing temperature) and a relative humidity of 70%.

**Treatment of *Drosophila* with selected diets:** For dietary restriction assays, different concentrations of Glucose (3g/100ml, 5g/100ml and 7g/100ml), vitamin C (0.25mg/100ml, 0.5mg/100ml and 0.75mg/100ml) and both Glucose plus vitamin were formulated in the diet. The appropriate concentration of nutritional composition is mentioned in the Table 1.

**Lifespan experiments:** For longevity analyses, flies were maintained in vials in groups of 10 (5 females and 5 males) and transferred into new vials every 4 days. We performed five independent experiments, using 100 newly eclosed flies for each experiment. In particular, we carried out this experiment using normal food (control media), treated media containing sucrose, vitamin and Glucose plus vitamin. Longevity was assessed using the modified protocol of Luckinbill and Clare, 1985.

## RESULTS

Fig. 1. The mean longevity of the dietary treated males and female flies. The flies fed with combination of Glucose plus Vitamin C resulted in an increased life span followed by Vitamin C and Glucose in both males and females when compared to control. However LD of Glucose fed flies have resulted significantly decreased lifespan when compared to control fed flies. Interestingly female flies have shown significantly increased life span in all the concentrations when compared to male flies.

## DISCUSSION

Nutritional environment is a potent mediator of an organism lifespan, in particular dietary restriction has been constantly found to extend lifespan across a vast range of animal taxa including Yeast (Lin *et al.*, 2002), Fruit flies (Chippidale *et al.*, 199), Mice (Weindruch and walford, 1982), Rhesus monkey (Roth *et al.*, 1999). The influence of distinct carbohydrates on ageing has previously been tested for several different model organisms, including the fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae). One of the pioneering studies in this field was performed by Hassett (Cho *et al.*, 2011). However, in Hassett's experiment, flies fed glucose solution had a slightly shorter life span than those on sucrose.

In several studies has been demonstrated that increased intake of protein may increase protein synthesis, decrease protein breakdown, reduce fat accumulation, and increase fat-free mass (Kerksick *et al.*, 2006; Piatti *et al.*, 1994) has been demonstrated. The flies fed with combination of Glucose plus Vitamin C resulted in an increased life span followed by Vitamin C and Glucose in both males and females when compared to control (Feg1). Moreover, separate labs often employ divergent diet-restriction protocols involving different levels of nutrient dilution and alteration of dietary components. Fitness is multifaceted thing and the relative contributions of different traits to fitness vary in different environments and contexts. Life history evolution and population dynamics are fundamentally linked because formal life-history theory developed out of models of population growth in age-related populations (Gadgil and Bossert, 1970; Charlesworth, 1994), and moreover, life history traits like survivorship and fecundity and their sensitivity to density, are the major determinants of population dynamics (Mueller *et al.*, 2000).

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