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LEVELS OF CARBOHYDRATES IN SHORT-LIVED STRAIN OF *Drosophila melanogaster*

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It is well known that the life expectancy of an organism is determined by the content of certain metabolites and activity of metabolic pathways [1]. Varied experiments have shown that reducing the protein content in the diet prolongs the lifespan of yeast, nematodes, and fruit flies. It is known that the glycolytic flux affects life expectancy: an accelerated glycolytic flux contributes to a shorter life expectancy, and vice versa. The concentration of certain glycolysis metabolites determines the rate of glycolytic flux and as a result, life expectancy changes [2].

Many studies have described that some epigenetic changes might be evolved to more rapidly detect and shape responses to changes in nutrient availability [3]. As a result, the action of the genes, affected by change of nutrient levels, may regulate the bioavailability of certain substances, or may act on metabolic pathways that are dependent on the availability of nutrients [4].

Aim. The aim of this study was to investigate the concentrations of key energetic substrates such as glucose, fructose, and trehalose, in control and artificially selected flies shown to have shorter lifespan.

Materials and Methods. For all experiments flies were maintained on standard yeast-sucrose nutrient medium consisted of 5% sucrose, 5% yeast, 1% agar and 0.18% nipagin as mold growth inhibitor [5]. Generation of experimental flies was previously described [6]. Flies of control w^{1118} and selected strains were maintained for about 100 generations after selection protocol was completed. Newly enclosed flies were kept two days for mating and used for measurement of feeding behavior and fecundity. Some flies were frozen at −80 °C for further analysis. Concentrations of glucose, fructose and trehalose were measured by Gas Chromatography/Mass Spectrometry using described protocol and expressed per wet weight. The columns were TR-5 MS with 30 m in length and 25 mm in diameter using 5% phenyl polysilphenylene-siloxane as stationary phase and helium as a carrier gas [7].

Results and Discussion. Our initial experiments showed that offspring of artificially selected flies have reduced lifespan for about 20%. To understand why these flies are short lived we evaluated feeding behavior and reproduction of females. Interestingly, control and experimental flies consumed virtually the same amount food both males and females. Moreover, female flies tend to lay slightly less eggs. Similar effects we have observed in our early work that showed no direct association between lifespan and consumption of macronutrients or reproduction [8]. Thus, we asked whether these flies might have different flux of metabolic pathways including glycolysis as an initial step of energy generation from carbohydrates.

Amount of glucose was significantly higher in experimental flies of both sexes (Figure). Male flies had about 70% more free glucose (Fig. *A*) and females for about 45% (Fig. *B*). These differences were accompanied with higher amounts of trehalose that consist of two molecules of glucose and is a form of glucose storage in Drosophila. Trehalose levels are dynamically regulated during development, impacting growth and metamorphosis. Its metabolism also may play role in gut microbiota interactions [9]. Trehalose content in experimental males was 2-fold higher, while in females it was higher for about 1.8-fold (Fig. *C* and *D*).

Interestingly, fructose was observed to be higher in experimental flies. This carbohydrate is metabolized through glycolysis, providing energy in the form of ATP. Beyond energy production,

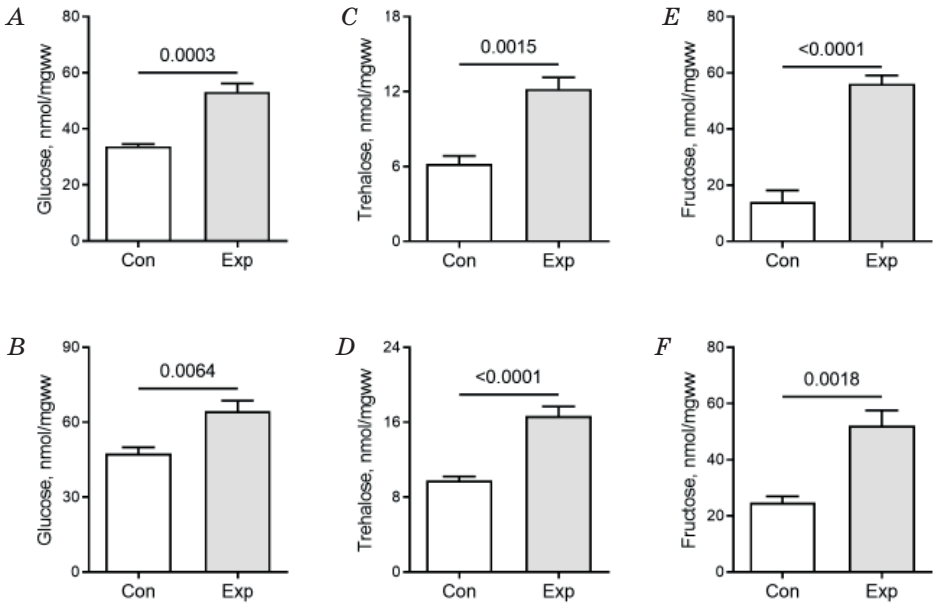

Fig. **Amounts of glucose (*A*, *B*), trehalose (*C*, *D*) or fructose (*E*, *F*) in control and offspring of artificially selected flies:**
A, *C* and *E* — males; *B*, *D* and *F* — females. Data are shown as mean SEM for 5–7 independent replicates

fructose plays a role in supporting reproductive processes such as oogenesis in females, though measure of fecundity showed no difference. Additionally, its metabolism may influence interactions with gut microbiota and contribute to the synthesis of storage carbohydrates like trehalose. The higher fructose content for about 4-fold was found in males and for about 2-fold in females (Fig. *E* and *F*). Consumption of both glucose, fructose or their mix was earlier shown to be beneficial for fly lifespan as compared to complex carbohydrates [10].

Conclusions. Our results show that despite similar consumption of food and reproduction offspring of artificially selected flies have shorter lifespan. However, this effect can be explained by lower energetic status while flies store more energetic substrates such as glucose, fructose and trehalose.

Key words: *Drosophila melanogaster*, nutrition, glycolytic metabolites, aging.

Authors' contribution. VVB and ISY performed sample preparation, evaluation of physiological parameters and metabolites, data analysis. OVL managed the project and wrote the manuscript.

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