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Specific Dietary Carbohydrates Differentially Influence the Life Span and Fecundity of *Drosophila melanogaster*

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The fruit fly, *Drosophila melanogaster* is a broadly used model for gerontological research. Many studies are dedicated to understanding nutritional effects on ageing; however, the influence of dietary carbohydrate type and dosage is still poorly understood. We show that among three carbohydrates tested, fructose, glucose, and sucrose, the latter decreased life span by 13%–27%, being present in concentrations of 2%–20% in the diet. Life-span shortening by sucrose was accompanied by an increase in age-independent mortality. Sucrose also dramatically decreased the fecundity of the flies. The differences in life span and fecundity were determined to be unrelated to differential carbohydrate ingestion. The highest mitochondrial protein density was observed in flies fed sucrose-containing diet. However, this parameter was not affected by carbohydrate amount in the diet. Fly sensitivity to oxidative stress, induced by menadione, was increased in aged flies and was slightly affected by type and concentration of carbohydrate. In general, it has been demonstrated that sucrose, commonly used in recipes of *Drosophila* laboratory food, may shorten life span and lower egg-laying capability on the diets with very low protein content.

Key Words: Carbohydrate diet—Sucrose—*Drosophila melanogaster*—Life span—Fecundity.

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CARBOHYDRATES are important dietary components for many omnivorous and herbivorous animals, including both humans and livestock. Carbohydrates provide energy for many reactions and processes flowing inside cells. Most organisms can tightly adjust their metabolism according to the availability of dietary components, including carbohydrates. Physiological effects of carbohydrates depend on their type and dosage, as well as on the physiological state of an organism (1). Very low carbohydrate intake restricts an organism's available energy and may slow down growth and regeneration, thereby altering survival and health. However, low carbohydrate intake has been proposed as a possible intervention to decrease the risk of, and complications related to, metabolic diseases such as obesity and metabolic syndrome (2,3). Many carbohydrates induce changes in metabolism and development of diseases when overconsumed. It was shown that fructose in high doses may lead to obesity and metabolic syndrome (4), high intake of glucose compromises the health of diabetic patients (5), and galactose can accelerate ageing (6). Many mechanisms may account for the toxicity of carbohydrates at overconsumption. Diabetic complications are mainly associated with glycation (7), the nonenzymatic reaction between the carbonyl group of the carbohydrate and amino groups of proteins or aminophospholipids. Products of glycation reactions can trigger the production of reactive oxygen species by binding special receptors (8). Excessive carbohydrates, when acting as signaling molecules, may change the overall metabolic balance by affecting lipid and protein biosynthesis (8,9). Nevertheless, the contribution of particular carbohydrates to specific pathological states remains a hotly debated topic, with some reports remaining controversial (5,10).

Many studies that examined the effects of carbohydrates on health have been focused on humans, as well as mouse or rat models. Generally, these effects were examined in relation to a preexisting condition, such as type II diabetes, obesity, metabolic syndrome, or hypertension. However, the effect of carbohydrate diets, and particularly the type of carbohydrate, as well as the protein-to-carbohydrate ratio on life span and reproduction are poorly investigated. They are generally studied in comparatively simple organisms like *Drosophila melanogaster*, which is intensively used as a model for nutritional studies. Over the last decade, several studies explored the effect of diet on life span, reproduction, behavior, and adaptation of fruit flies (11–13).

Carbohydrates in fruits, as consumed by *Drosophila* species, as well as by humans, are present as a mixture of fructose, glucose, sucrose, and other carbohydrates (14,15). Average concentrations of these carbohydrates in fruits are around 6%–16% (15). The processed food of contemporary human populations is usually skewed toward one or another

carbohydrate and, in the case of high fructose corn syrup, is often provided in excess of natural percentages (16,17). It has been demonstrated in both human communities and animal models that diet can influence life span (11,18). Thus, it is perhaps not surprising that the type and the dosage of carbohydrate, as well as its proportion to other dietary components may affect life span. Taking into account that *Drosophila*'s natural food sources are generally not as rich in proteins as are the diets found in laboratory cultures, in our previous study (19) on fruit fly as a model, we used diets with relatively low amounts of protein and different concentrations of sucrose as a carbohydrate source to assess the role of macronutrient balance on life span and functional senescence. The life spans obtained were comparable with those observed in other studies where the fruit flies were maintained on higher protein levels (20,21). The important factor was determined to be the ratio between protein and carbohydrate levels. Using the same recipe in the present study, we have evaluated the influence of different carbohydrates such as fructose, glucose, and sucrose on the life span and reproduction of fruit flies. We have also tested the flies for their ability to survive oxidative stress at different ages.

Materials and Methods

Fly Stock and Husbandry

Inbred line of *D melanogaster* (called "IF") was used in the study. Parental flies were originally collected in the southeast part of Ivano-Frankivsk (Western Ukraine) in 2007 and maintained for more than 30 generations on yeast–molasses medium at 25°C, under a 12:12 light:dark cycle, and 60%–70% relative humidity.

Life-Span Studies

Parental flies were reared in 250-mL bottles with 25 mL of medium containing 6% (w/v) yeast, 4% (w/v) molasses, and 0.4% propionic acid. Routinely, 200–250 eggs were placed in each bottle. Newly eclosed flies were transferred to fresh medium for 2 days to mate. Then, they were separated by gender and transferred without anesthesia in groups of 10 to glass vials (15 × 150 mm) with 1.25 mL of fresh experimental medium. Experimental media contained 0.25% yeast extract (Sigma-Aldrich, 70161) as a source of protein and micronutrients, 0.4% propionic acid as a mold inhibitor, 1% of agar, and appropriate carbohydrate in concentrations of 0.25%, 0.5%, 2%, 4%, 6%, 10%, and 20%. Carbohydrates used were fructose, glucose, sucrose, and a mixture of fructose and glucose at 1:1 ratio. Food was changed every second day and the number of dead flies was counted. To minimize any density effects on mortality, two vials with cohorts were merged when the density of flies reached five or less individuals. To standardize the effects of parental age on offspring fitness, parents of experimental flies were of the same age (4–5 days) and reared at a constant density for at least two generations. Three independent trials with about 100 flies per diet and sex were performed. The life span curves for every diet and sex represent cumulative survival for about 300 flies and Kaplan–Meier survival analysis was performed. The difference in survival between cohorts was assessed by log-rank test using JMP 9.0 statistical software (SAS Institute). Mean life span was calculated as average survival in cohorts. Mean life span was compared by Student's *t* test. Age-specific mortality (μ_x) was estimated as $\mu_x = -\ln(p_x)$, where p_x is the probability of an individual, alive at age $x - 1$, survive to age x.

Survival curves were fitted by the equation $N_t = N_0 \exp\{A(1 - \exp(\alpha t))/\alpha\}$ (22), where N_t is the number of alive individuals at any time moment, N_0 is the initial cohort size, and A and α are age-independent and age-dependent parameters of Gompertz equation, respectively. The package *minpack.lm* for R software (version 3.0.1) was used for survival curve fitting and calculation of Gompertz equation estimates.

Fecundity Test

Once mated females were reared in small vials (15×60 mm) with 0.7 mL of experimental food. Food was changed every day. The number of eggs laid by individual females was then counted. For the diets with 2%–20% carbohydrates, measurements were performed during the first 32 days of adult life. Eggs were counted every second day. For the diets with lower carbohydrate concentrations, the measurement lasted a week, and counts were performed every day. The differences between groups of 8–25 flies were calculated with Student's *t* test.

Determination of Feeding Rate

Food intake by a single fly was measured as described in Lee and colleagues (11). Briefly, 3-day-old single flies were transferred to 1.5-mL vials with a small piece of filter paper and 5-µL capillary tubes (Drummond) filled with liquid food (as described for life span but without the agar). Vials were kept within closed boxes with a thin water layer on the bottom to maintain high humidity. The capillary tubes were replaced each day and the amount of food consumed was measured more than 4 days. Eight flies were tested in parallel, per diet and sex. The test was performed in two replicates with 50–75 individual flies in each. Sixteen vials without flies were used as controls for each diet treatment to subtract volume of liquid evaporated from the top of the capillary.

Menadione Treatment

Male and female flies were fed with diets containing 6% or 10% of carbohydrates in same conditions as for life span assays. Every 10th day, 10 flies from each of the diets

were transferred to empty vials for 4 hours to starve. After starvation, flies were put into vials containing folded and compacted strips (2.4×12 cm) of 4-layer cellulose filter paper soaked with 0.8 mL of 20 mM menadione sodium bisulfite (Sigma-Aldrich, M5750) in 5% sucrose solution. Survivors were counted after 24 hours of exposure and groups were compared with Fisher exact test. To check if menadione induced oxidative stress the activities of antioxidant enzymes such as superoxide dismutase and catalase, as well as sensitive to oxidation enzyme aconitase, were evaluated according to Lushchak and colleagues (23).

Mitochondrial Protein Density

Mitochondria were isolated as previously described by Fernandez-Ayala and colleagues (24). Mitochondrial protein density (MPD) was measured as a ratio of citrate synthase activity in whole fly homogenate and isolated mitochondria according to Magwere and colleagues (25). Briefly, citrate synthase activity was measured by colorimetric estimation of absorbance at 414 nm of conjugate formed between 5,5′-dithiobis (2-nitrobenzoic acid) and acetyl coenzyme A in the reaction mixture containing 90 mM Tris-HCl (pH 7.5), 0.5 mM ethylenediaminetetraacetic acid, 0.3 mM acetyl coenzyme A, 0.1 mM 5,5′-dithiobis (2-nitrobenzoic acid), and 0.5 mM oxaloacetate. The blanks without oxaloacetate were used to each sample for exception of nonenzymatic reaction between 5,5′-dithiobis (2-nitrobenzoic acid) and thiol compounds in homogenate and isolated mitochondria.

Results

Life Span and Estimates of Gompertz Equation

Fruit fly females, on a diet of 2%–20% sucrose, had mean life spans shortened by 13%–27% in comparison with those that consumed food containing fructose or glucose in the same concentration range (Figure 1 and Supplementary Table S1). Similar results were obtained for males (Supplementary Figure S1). There was no significant difference in life span between fruit fly cohorts fed diets with 0.25% and 0.5% carbohydrates.

Survival analysis by Gompertz equation showed that the individuals kept on diets containing 2%–20% sucrose had higher age-independent mortality (Figure 2) compared with those fed on other sugars, whereas age-dependent mortality

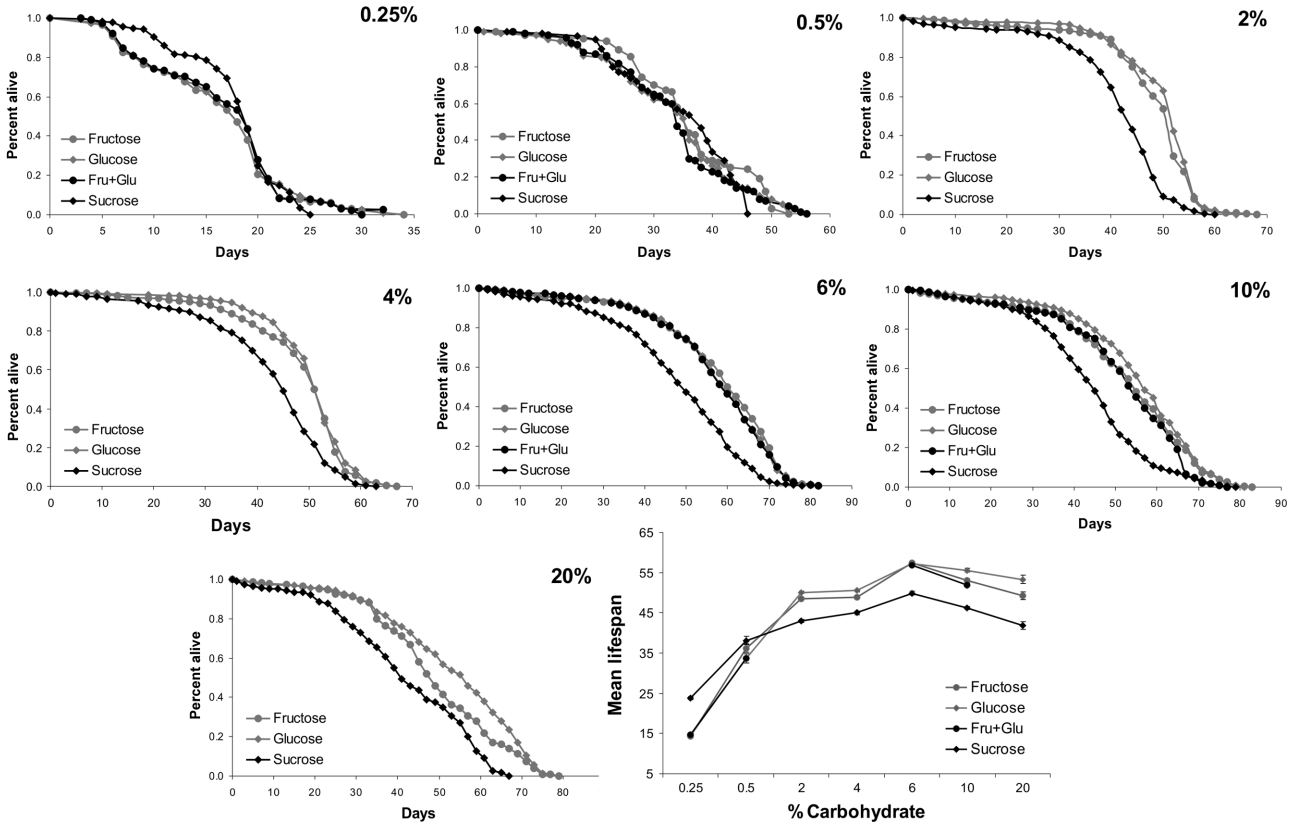

Figure 1. The type and concentration of carbohydrate in the food altered the life span of female fruit flies. Diets with fructose, glucose, and sucrose in concentrations of 0.25%, 0.5%, 2%, 4%, 6%, 10%, and 20% were prepared by the mixing of yeast extract (0.25%) and different concentrations of carbohydrates. Equimolar mixture of fructose and glucose was used to mimic sucrose at 0.25%, 0.5%, 6%, and 10%. Mean life span represents time (in days) survived by average fly in cohort. Each curve represents percentage of individuals alive as a function of age for about 300 flies. Differences between carbohydrates are also given in Supplementary Table S1.

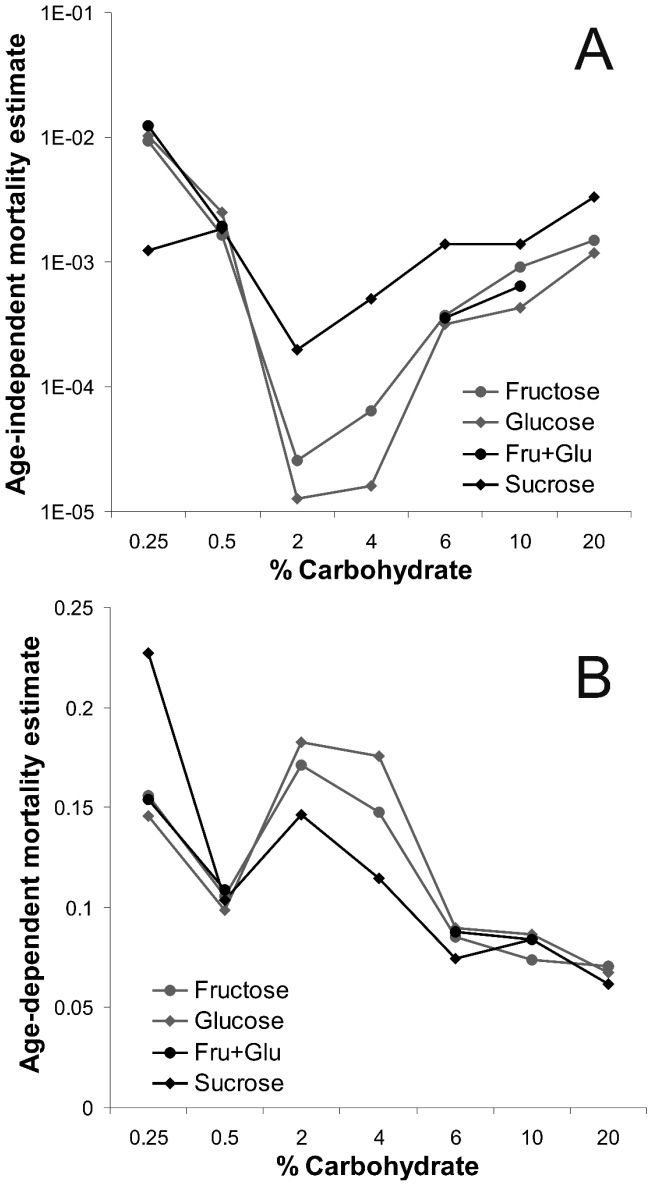

Figure 2. Sucrose decreased life span increasing age-independent mortality. Age-independent (**A**) and age-dependent mortality (**B**) were defined by fitting the survival curves with modified Gompertz equation (see Materials and Methods section for description). Levenberg–Marquardt algorithm was used for minimization and calculation of the equation estimates. The accuracy of the fitting was evaluated by Student's *t* test ($p = .025$). For age-independent mortality estimates, standard deviations derived from the fitting procedure were <30% of the fitted value, except those for diets with 2% fructose and glucose, where standard deviations equaled 32.2% and 39.3%, respectively. For age-dependent mortality estimates, standard deviations were all <10%.

was slightly decreased in a range between 2% and 6% sucrose. Notably, the lowest value of age-independent mortality was observed at 2% carbohydrates for all diets. Both estimates were highest at 0.25% carbohydrate partially indicating starvation. Diets with 0.5% carbohydrates seem to be sufficient to support fruit fly existence for around 1.5 months with a relatively small concentration of protein.

Fecundity

Females fed diets with 2%–10% sucrose showed from three- to sixfold lower average egg-laying capability as compared with those fed other carbohydrates (Figure 3). Often, fecundity was approximately equal on all diets with 2%–10% carbohydrates during the first weeks after mating. Fecundity of flies on sucrose-containing medium decreased dramatically after the first weeks postmating from 8–12 to 0–2 per day and remained constantly at this level. For individuals consuming carbohydrates other than sucrose, age-dependent changes in egg laying during a period of 32 days showed oscillations with a period around 10–20 days (data not shown). The fecundity of flies on diets with 0.25% and 0.5% carbohydrates was decreased, perhaps due to a lack of macronutrients. On diets with 20%

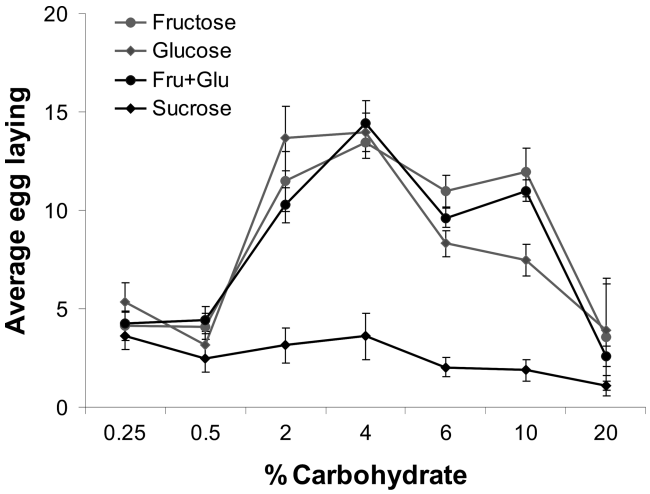

Figure 3. Food supplementation with sucrose dramatically decreased egg-laying capability. Average amounts of eggs laid by single females were quantified for a period of 32 days. Points represent amount of laid eggs ± standard error of mean. Values for individuals fed on diets with 2%, 4%, 6%, and 10% of either fructose, glucose, or equimolar mixture of fructose and glucose were significantly different (with $p < .05$ by Student's t test, $n = 8–25$) from corresponding values for sucrose-fed flies. Values for individuals fed on diets with 2%, 4%, 6%, and 10% of fructose and the mixture were also significantly different from corresponding values for flies consumed the same carbohydrates in concentrations 0.25%, 0.5%, and 20%, whereas for glucose-fed flies, only fecundity on 2% and 4% carbohydrate was significantly different from that on 0.25%, 0.5%, and 20%.

carbohydrates, fecundity drastically decreased with the age of the flies. Average fecundity at 20% carbohydrates was as low as at 0.25% and 0.5%, when sucrose was maintained at the lowest value tested.

Food Consumption

Amount of food consumed by the flies on the different diets was measured to evaluate whether compensatory feeding took place. In this case, the type of carbohydrate did not have a considerable influence on the amount of carbohydrate consumed (Figure 4, shown for the range 2%–10% carbohydrates). We did observe that flies consumed more food on low carbohydrate diets, likely in order to achieve an approximately equal carbohydrate intake. We fitted the relationship between consumed carbohydrates and their concentrations by the second-order polynomial equations (Supplementary Table S2). Interestingly, estimates of a, b, and c for fructose and glucose attained extreme positions, whereas those for the mixture of fructose and glucose and for sucrose dropped in between them. Parameter c for fructose and glucose mixture was found to be closer to that of fructose, whereas that for sucrose was closer to that for glucose. These results may suggest a differential perception of carbohydrates by the animals and, perhaps, represent the fruit flies response to distinct nutritional values between the carbohydrates, what we will explore further in the Discussion section.

Resistance to Oxidative Stress

Resistance to menadione, a redox-cycling agent that generates superoxide anion radicals, was measured from cohorts living on diets with distinct carbohydrates at 6% or 10% of their total food. Measurements were performed until Day 30 (52%–68% of the mean life-span values) of the lifetime of adult fruit flies. An induction of oxidative stress by menadione was confirmed by measurement of activities of main antioxidant enzymes, superoxide dismutase and catalase, as well as the activity of aconitase, which is considered to be sensitive to oxidation by superoxide (26). Treatment of fruit flies with menadione induced a twofold increase in superoxide dismutase activity ($p = .0347$ by t test), whereas resulting in a decrease in aconitase activity ($p = .0129$; Supplementary Figure S2).

In general, males fed on the diets with a higher carbohydrate concentration had lower resistance to menadione except for those on sucrose diets (Figure 5). Particularly, diets with 10% fructose and glucose decreased menadione resistance of 30-day-old males 2.7- and 12.7-fold, respectively, as compared with diets with 6% of these carbohydrates ($p = 5.92 \times 10^{-4}$ for fructose and 1.28×10^{-8} for glucose by Fisher exact test). However, males that lived on a 10% equimolar mixture of fructose and glucose, and 10% sucrose, showed resistance similar to that on 6% carbohydrate diet ($p = .37$ for the mixture and .31 for sucrose by Fisher exact test). After menadione treatment, only 20% of the 30-day-old males on 6% sucrose survived. At the same time point, flies on fructose, glucose, and equimolar mixture of fructose and glucose of the same concentration showed 3.1-, 3.2-, and 2.8-fold higher survival, respectively ($p = 2.24 \times 10^{-4}$, 9.23×10^{-5}, and 2.40×10^{-3}).

Resistance to menadione in female cohorts did not show significant dependency on carbohydrate concentration. Menadione treatment during 24 hours caused death of 63%–73% individuals among 30-day-old females on

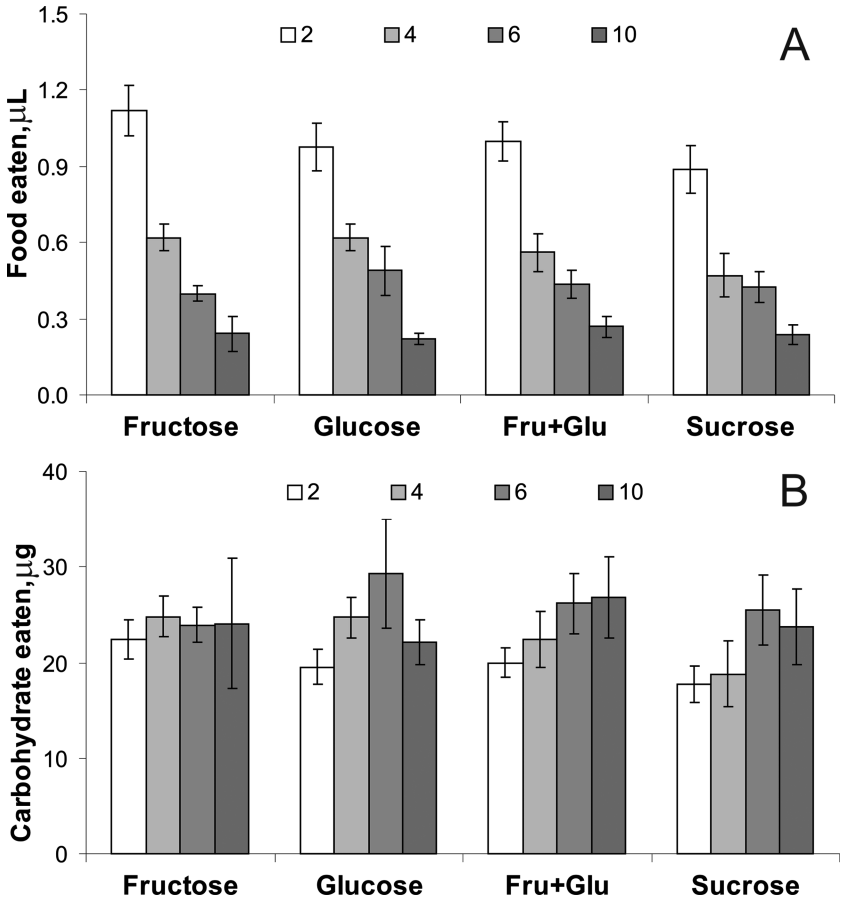

Figure 4. Flies consumed different volumes of food (**A**) on different carbohydrates, but the absolute amounts of carbohydrate (**B**) were approximately equal. Bars represent mean ingestion by single females (volume in **A** or amount of carbohydrate in **B**) ± standard error of mean for 32 flies for a period of 4 days. Values for food eaten by flies on diets with 2% carbohydrates were significantly different (with $p < .05$ by Student's t test, $n = 115–124$).

diets of 6% carbohydrates. Diets with higher carbohydrate concentration conferred slightly higher resistance. The highest resistance was observed for females fed a diet of 10% glucose. Survival of these flies was 1.6- to 1.8-fold higher than that of those on other carbohydrates at the same concentration.

Mitochondrial Protein Density

The MPD was measured in flies fed diets with 0.25% and 10% of carbohydrates for 10 days. The method used is based on the measurement of citrate synthase activity in whole fly homogenates and purified mitochondria. MPDs of 0.474 and 0.379 were observed in flies fed diets with 0.25% and 10% of glucose, respectively (Figure 6). Replacement of glucose by fructose in the diet significantly increased the MPD by 34% at a carbohydrate concentration of 0.25% ($p = 2.11 \times 10^{-2}$) and by 76% at 10% carbohydrate ($p = 2.84 \times 10^{-3}$). Finally, the MPD was highest in flies fed the diets with sucrose, differing by 22% and 63% from fructose and glucose, respectively, at 0.25% carbohydrates ($p = 2.85 \times 10^{-2}$ and 4.33×10^{-4} for t test) and by 25% and 120% at 10% carbohydrates ($p = 3.90 \times 10^{-2}$ and 7.75×10^{-4}).

Discussion

The influence of distinct carbohydrates on ageing has previously been tested for several different model organisms, including the fruit fly, *D melanogaster*. One of the pioneering studies in this field was performed by Hassett (27). In general, our conditions closely resembled that report. We used seven concentrations representing the range from 0.014 M to 1.11 M for fructose and glucose, and from 0.007 M to 0.584 M for sucrose. Comparable concentrations in Hassett's report are close to those in our diets containing 2% carbohydrates. Our results are partially in agreement with Hassett's conclusions because our flies, consuming fructose, lived longer than did those ones consuming sucrose (Figure 1). However, in Hassett's experiment, flies fed glucose solution had a slightly shorter life span than those on sucrose. In our case, there was no significant difference in life span between fructose- and glucose-fed fly cohorts, whereas in sucrose-fed flies, life span was significantly shorter. Of note, in Hassett's study, the death of the last fly on the diets containing 1.7%–1.8% carbohydrates was observed at around 30th day of adult fruit fly lifetime, whereas in our study, flies lived at these

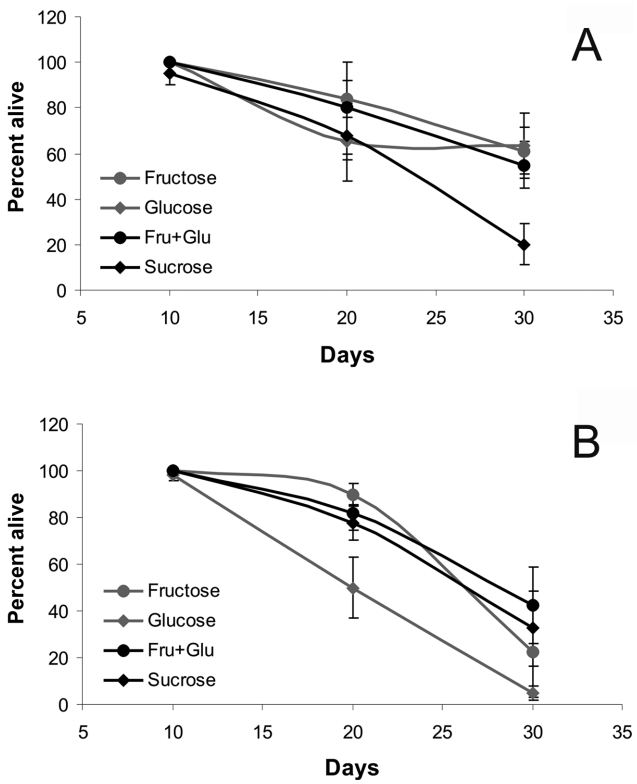

Figure 5. Sensitivity toward oxidative stress increases with age in males flies fed carbohydrates in concentrations of 6% (**A**) or 10% (**B**). At ages of 10, 20, and 30 days, flies were transferred to test tubes with 20 mM of menadione in 5% sucrose. Alive flies were counted after 24 hours. Values and standard error of means were defined from three to four independent measurements for 8–10 flies per each sex/day/diet.

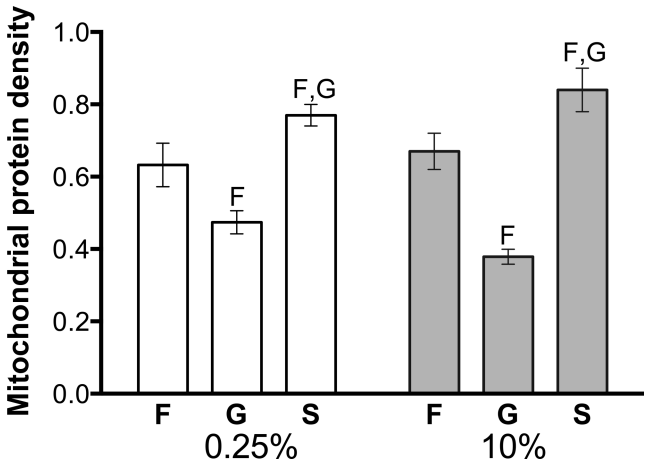

Figure 6. Type of carbohydrate rather than concentration affects mitochondrial protein density. Flies were kept for 10 days on the diets with 0.25% or 10% of carbohydrates. Mitochondrial protein density was quantified by citrate synthase activity determined in homogenates from fruit flies and in its mitochondrial fractions. The values are represented as ratios of whole fly and mitochondrial fractions. Data are shown as mean values ± standard error of mean.

concentrations-more than 60 days. This implies possible starvation conditions in Hassett's experiments. Other possible cause for the observed differences may be fly strain background and a difference in humidity conditions, which affects the life span of flies.

Gompertz equation estimates, *A* and α, age-independent and age-dependent mortalies, respectively were highest in our case for flies on diets with 0.25% carbohydrates (Figure 2). This suggests an influence of stressful conditions, most likely, starvation. Thus, lack of nutrients may cause the death of vulnerable individuals at early age, whereas the harm from starvation lead to advanced mortality of more resistant flies. Noteworthy, α for female cohorts fed 0.25% sucrose was higher than that for cohorts fed

other carbohydrates. This means that low sucrose diet may augment starvation stress and promote the death of aged individuals.

The role of mitochondria in regulation of *Drosophila* life span was extensively studied and summarized by Cho and colleagues (28). We have measured the MPD in flies fed diets with 0.25% or 10% of carbohydrates. Of note, life spans were virtually the same in flies that consumed a 0.25% carbohydrate diet, whereas a shorter life span was observed for flies fed a diet with 10% sucrose compared with those that consumed fructose, glucose, or equimolar mixture of fructose and glucose (Figure 1). We did not find any correlation between life span and MPD. Flies fed diets with 0.25% carbohydrates had almost the same MPD as flies fed with 10% carbohydrate diet, but their life span was significantly shorter. This fact clearly shows that the difference in life spans between flies is not linked to MPD. MPD fails to explain why at carbohydrate concentration of 0.25% there was no difference in fly life spans but at 10% carbohydrate, flies fed sucrose diet lived shorter. It was shown also that MPD was not changed by dietary restriction (25) and rather changes in mitochondrial function and activity of electron transport chain were proposed to be the main factors of mitochondrial effects on life span (28). However, it seems that MPD depends on the type of carbohydrate. Particularly, fructose and sucrose promote higher densities than glucose. Recently, it was shown on mice that fructose can stimulate adenosine monophosphate-activated protein kinase (29,30). In turn, adenosine monophosphate-activated protein kinase can activate peroxisome proliferator-activated receptor-γ coactivator-1α involved in mitochondrial biogenesis by coactivating corresponding transcriptional factors, such as nuclear respiratory factor 1, nuclear respiratory factor 2, peroxisome proliferator-activated receptor gamma, and others (31).

Our data show considerable restriction in the egg-laying capability of fruit flies fed sucrose-containing diets (Figure 3). The same result was observed in earlier studies for flies reared on high sucrose media (32,33), though the emphasis of the studies was placed on biochemical traits, the influence of selection (32), and developmental hormone production (33). In particular, it was shown that a sugar diet combined with a deficit of macro- and micronutrients can lead to a dramatic decrease in yolk protein concentration in adult female flies (33). Nevertheless, in the latter study, only the sucrose effect was evaluated. In our case, females showed relatively low egg-laying capability on every carbohydrate diet, perhaps due to protein deficiency. However, sucrose-fed females showed extremely low egg-laying capability in comparison with flies fed with other carbohydrates. At the same time, flies on a diet containing a mixture of fructose and glucose showed an egg-laying capability comparable with that of flies on a diet of either fructose or glucose. Thus, sucrose metabolism may require more energy under protein restriction.

Interestingly, Bass and colleagues (34) have also found that sucrose at high concentrations decreases egg-laying capability, but unlike in our case, they did not find a corresponding decrease in life span. However, a shortening of both life span and reproduction by sucrose was clearly demonstrated for the pea aphid (35). As with our study, the protein concentration in the latter study was restricted, and insects were fed with amino acid cocktail. Taken together, these data suggest that at relatively high dietary protein levels, the effects of sucrose on life span may be negligible, but the adverse effects on egg laying remain.

Analysis of feeding (Figure 4 and Supplementary Table S2) shows that food consumption depends on carbohydrate concentration in a certain range and this dependency can be fitted to a polynomial regression equation. The same was observed for the amount of consumed protein. Food consumption can be affected by several parameters, including food perception and satiety. The first parameter is likely to be conferred by carbohydrate type, as *D melanogaster* can reportedly distinguish different carbohydrates by sensory perception (36). In turn, satiety is defined by metabolite concentrations (30) and is mediated by neuropeptide signaling (37). In our previous studies (19,23), we have shown that both *Drosophila* larvae and adult flies try to compensate for a deficit of carbohydrate in their food by increasing consumption. The same phenomenon was observed by other groups using different methods (38,39). For comparison, in our previous experiments, flies on sucrose-containing diets consumed about 60–70 μg of carbohydrates per day as measured by the CAFE assay (19). In the present study, we can see approximately 1.5-fold less carbohydrate ingestion, which may depend on the season in which the assays were conducted. Indeed, seasonal differences in metabolism were found for the closely related species *Drosophila simulans* (40). Values of carbohydrate intake as measured here were also comparable with the data of Vigne and Frelin (41). Interestingly, their flies were kept on diets with much higher protein concentrations. This implies that food intake may depend predominantly on the carbohydrate concentration. It is clear from this study and our previously reported work that flies attempt to compensate for a carbohydrate deficit, while a lack of protein has relatively weak influence on their life span.

Notably, fructose-fed flies consumed slightly more food on a diet with 2% carbohydrates. Similar, but more striking differences we observed for larvae with the colored food assay (23) and for adult flies with the CAFE assay (19). It suggests that fruit flies may have a higher threshold for satiety at relatively low fructose concentrations.

Because sucrose is abundant in natural fruit fly food and is a main component of laboratory media, including ours, sucrase activity is expected to be constitutive, and unlikely to be a "bottleneck point" in the restriction of life span and egg-laying capability by sucrose. However, sucrose, in

combination with low protein and micronutrient concentration, can also influence *Drosophila* gut microorganisms. There are several lines of evidence for this hypothesis. In particular, it was shown that bacteria can improve digestion of plant polysaccharides (42). Changes in the gut microflora of *Drosophila* could increase (43) or decrease mortality of the host (44). On the other hand, diet influences structure and species diversity of the symbiotic gut bacteria community in insects (45).

For the menadione resistance test, we had chosen diets on which fruit flies showed the longest life span in order to check the possibility of sucrose to decrease life span and fecundity by modulation of antioxidant defense. Prior to longitudinal studies on menadione resistance, we have tested if menadione is able to induce oxidative stress in vivo. The increase in superoxide dismutase activity and concomitant decrease in aconitase activity, the enzyme thought to be susceptible to the superoxide anion, clearly suggest an overproduction of this form of reactive oxygen species in fruit flies treated with menadione (Supplementary Figure S2). It has been shown that redox-cycling compounds, like paraquat, modify food consumption (46) and we tested it for menadione measuring consumption of food containing this redox-cycling agent. Flies ate less menadione-supplemented food in general but this decrease in food consumption was equal for the flies taken from different carbohydrate diets at 10th day of their lifetime (Supplementary Figure S2). Our previous studies, performed on *Drosophila* (23) and budding yeast *Saccharomyces cerevisiae* (47), as well as investigations of other teams, suggested that antioxidant defense can be modulated by both the carbohydrate type and concentration in the diet. Fructose, particularly at high concentrations, is capable of promoting the generation of reactive oxygen species (48). In the current experiment, we used a dietary preconditioning to the toxic doses of menadione. Thus, the results can be evaluated from different angles. On the one hand, the diet could make flies more vulnerable to the following oxidative stress if the prooxidative action of the carbohydrates is sufficiently strong and may then lead to systematic oxidative damage. On the other hand, dietary carbohydrates with moderate prooxidative activity could induce antioxidant defenses but not necessarily lead to oxidative damage. In the present case, the last scenario is more likely because the longest life spans were observed on the chosen diets, and the age-dependent mortality was relatively low except for flies on the sucrose-containing diet. However, menadione resistance data suggests that only males on 6% fructose and glucose may keep strong antioxidant defenses with age, what provide oxidative stress resistance to the considerable part of 30-day-old individuals. Aged males, kept on a diet with 10% of fructose or glucose, were more vulnerable to menadione compared with those kept on 6% carbohydrate diets. Thus, the effect of carbohydrate diet may not be observed by the analysis of the life-span curve, whereas resistance to stressors may reveal a potential of the diet to support functional adolescence or promote senescence. According to this, 10% carbohydrates in combination with a protein deficit seem to be a marginal concentration, and further increase may lead to the life-span shortening.

Conclusions

Results, described and discussed here, have shown that experiments using *D melanogaster* as a model for the investigation of life span should take into account both the type of carbohydrate and the protein-to-carbohydrate ratio of the flies diet, as well as the rate of food intake. Sucrose is commonly used in recipes of *Drosophila* laboratory food, but it may contribute to life-span shortening and lower egg-laying capability. Current information on sucrose metabolism in the fruit fly is scarce. Because *D melanogaster* is a model to study metabolism and ageing (49), further information clarifying the effects of dietary sugars would benefit the field as a whole. It should also be considered that carbohydrates may differ in their elicitation of satiety; hence, they can also modulate the rate of food intake.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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