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DEVELOPMENTAL DIET PARTIALLY DETERMINES AGE-RELATED CHANGES IN METABOLISM OF *DROSOPHILA*

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Abstract. Aging and metabolism are inextricably linked and both are critical for longevity. Changes in energy metabolism occur during normal aging and may be partly improved through the alteration of lifestyle variables. Macronutrients have a significant impact on life-history traits, such as disease vulnerability, reproduction, longevity and stress resistance. We investigated the age-related changes in the metabolism of fruit flies *Drosophila melanogaster* fed different diets at the developmental stage. Adult flies were transferred to the fresh two types of food. Two-, 15- or 40-day-old flies of every experimental group were tested. We observed significant changes in carbohydrate and fat metabolism associated with developmental diet and age. Developmental nutrition influenced hemolymph glucose and triglyceride (TAG) levels in young and adult flies. Hence, the consumption of a fixed diet during a long period of the adult stage can equalize the impact of a developmental diet. Higher hemolymph glucose level was observed in flies fed by a medium composed of 5% yeast as compared to a Yeast-Molasses medium during development. However, higher TAG and lipid pool were associated with consumption of Yeast-Molasses medium during development. Carbohydrate storage declined with the simultaneous increase in lipid pool with age. The impact of developmental nutrition and age on carbohydrate and lipid metabolism are not associated with gender or nutrition during adult life. Insulin and insulin-growth-factor-like signaling and target of rapamycin (TOR) pathways may be considered as the potential mechanisms involved in the regulation of developmental pathways by dietary conditions. Our data clearly show the connection between nutritional conditions during development and metabolic alteration with aging in *Drosophila*.

Keywords: aging; metabolism; carbohydrates; fruit fly; development.

1. INTRODUCTION

Aging is a complex process of progressive decline in physiological integrity and function that can be affected by genetic and environmental factors. Nutrition is one of the most significant environmental factors that influence aging through modulation of metabolism. Diet experienced during development has a profound impact on organismal health and physiology in adult life. The adverse intrauterine environment led to changes in the metabolic profile and induced metabolic syndrome in adult offspring (Vaiserman et al., 2018). Different types of stresses, such as starvation or lack of nutrients at certain stages of development, cause epigenetic changes, which reproduce over the course of many mitotic cycles, causing long-term changes in various processes and structures (Magalhães et al., 2012).

Fruit flies *Drosophila melanogaster* is a suitable model organism for studying aging mechanisms because of its unique properties including relatively small body size, a very rapid life cycle and a short lifespan. It was shown that developmental diets with low yeast or high sugar content led to reduced reproductive capacity and body size in flies regardless of adult diet (Klepsatel et al., 2020; Strilbytska et al., 2022a). At the same time, larval underfeeding strongly affected lifespan in *Drosophila* (May et al., 2015). Diet supplemented with either fructose or glucose during development

had distinct effects on free radical processes and activity of antioxidant enzymes in adult flies (Lushchak et al., 2011). We have recently investigated the influence of larval diet on developmental rate and metabolism in larvae and imago (Strilbytska et al., 2022a). The Developmental Origins of Health and Disease hypothesis developed by Baker and colleagues (Barker et al., 1993) predicts that early-life environmental exposures can be detrimental to later-life health. We aimed to compare the metabolite levels in young (2-day-old), adult (15-day-old) and old (40-day-old) flies reared on two types of diets during the developmental stage. We found different patterns of changes in metabolites in response to age. Moreover, metabolite levels at a certain age were strongly dependent on developmental diet. Our results help to partially explain the alterations of carbohydrate and fat metabolism occurring with age, and the relationship between nutritional factors and metabolic changes.

2. MATERIALS AND METHODS

Fly strain and husbandry

Wild type *Canton-S* flies [*D. melanogaster* Meigen] were used in all experiments. Stock flies were provided by Bloomington Stock Center (Indiana University, USA). Flies were maintained at 25°C under a relative humidity of 60-70% and 12:12 hours day/night cycle. The parental population of flies was maintained on a yeast-molasses diet (Y-M) containing 7.5% (v/v) molasses, 5% (w/v) dry yeast, 6.1% (w/v) corn, 1.2% (w/v) agar, 0.3% (v/v) propionic acid and 0.18% (v/v) nipagin as anti-fungal and anti-bacterial agents (Lozinsky et al., 2012).

Experimental procedures

Flies aged 3-7 days were subjected to 3-hour starvation with subsequent 15-hour eggs-laying on the diet composed of 5% sucrose and 2% agar with the addition of dry yeast on the surface of the medium. To prevent effects caused by larvae density, laid eggs were washed three times with distilled water, then concentrated and about 100 eggs were transferred into bottles containing 25 ml of experimental diet. Larvae were allowed to develop on two types of diets – 5Y (5% of dry yeast and 1.2% of agar) or Yeast-Molasses (Y-M described earlier). Eclosed flies were transferred to the fresh medium and kept for two days for mating. Next, flies were separated by sex and transferred on the fresh two types of food 5S-5Y (5% sucrose, 5% dry yeast and 1.2% of agar) or 20S-2Y (20% sucrose, 2% dry yeast and 1.2% agar). Experimental food was changed every other day. Two-, 15- or 40-day-old flies of every experimental group (I-IV; see Table 1) were frozen in liquid nitrogen for further biochemical assays.

Table 1. Description of groups in according to nutritional regimes during development and adult stage.

	Diet during development	**Diet after eclosion**
Group I	5Y	5S-5Y
Group II	Y-M	5S-5Y
Group III	5Y	20S-2Y
Group IV	Y-M	20S-2Y

Fly weight

Female and male flies were weighted in groups of 20 with a precise balance WTW 2 ("Techniprot," Poland). Then flies were transferred into ventilated vials and kept in the thermostat at 60°C for 48 hours. Dried flies were weighed again for the determination of dry body mass.

Hemolymph extraction

Anesthetized flies were punctured in the thoracic segment of the body and placed in a plastic tube (0.5 ml with a hole at the bottom) embedded in a larger tube (1.5 ml). Centrifugation was performed at 7000 rpm for 8 min at room temperature. Hemolymph was collected and dithiothreitol was added at a concentration of 0.15% with subsequent centrifugation (6000 g, 15 min, 4°C). The diluted hemolymph was heated at 70°C for 5 min and centrifugated (16000 g, 15 min, 4°C) to remove proteins. The hemolymph was transferred into clean plastic tubes and stored at -18 °C until measurements (Semaniuk et al., 2018).

Glucose and glycogen assay

Weighted fly bodies were homogenized in 50 mM sodium phosphate buffer at a ratio 1:10 (weight/volume), centrifuged (16000 g, 15 min, 4°C) and used for determination of glucose and glycogen levels. The supernatants were heat-denatured as was described previously (Strilbytska et al., 2022). Measurements were conducted using a glucose assay kit (Liquick Cor-Glucose diagnostic kit, Cormay, Poland, Cat. #2-203) following the manufacturer's instructions. Glycogen was converted into glucose by incubation with 1.5 mU/μl amyloglucosidase from Aspergillus niger (Sigma-Aldrich Chemie GmbH, #10115) for 18 hours at 37°C. The level of glycogen was calculated by subtracting glucose amounts. Absorbance was determined at 500 nm using Specol-211 spectrophotometer (Germany) (Semaniuk et al., 2018).

Triglyceride levels determination

Weighted 6 flies of each cohort were homogenized in PBS buffer (pH 7.4), at a ratio 1:34 (number of flies:μl PBS). Homogenates were boiled and centrifuged at 13000 g, for 10 minutes at 21°C. Liquick Cor-TG diagnostic kit was used to determine triacylglyceride content in supernatants (Cormay, Poland, Cat. #2-254). Optical density was determined at 550 nm on a Specoll-211 (Strilbytska et al., 2020).

Assay of total lipids

Total lipid concentration was determined using a TL kit (Erba Lachema, Czech Republic). Frozen flies were weighed and homogenized in 96% cold (4°C) ethanol (1:15 w:v). The homogenates (75 μl) were mixed with an equal volume of chloroform and centrifuged (800 g, 2 min, 21°C). Vials with 40 μl of supernatant were stored on ice until all samples had been evaporated to dryness. Dry samples were incubated in a water bath (95°C) for 20 min with 200 μl of concentrated H2SO4. Subsequently, 200 μl of kit reagent and 1 ml concentrated H_2SO_4 were added to the cooled samples for a total volume 1.4 ml. Absorbance was determined spectrophotometrically at 440 nm.

Statistical analysis

Data are presented as mean ± SEM and analyzed using Two-way ANOVA followed by Holm-Sidak's (H-S) test. Differences between groups were considered statistically significant when $p < 0.05$. Graphing and statistical analysis were performed by using GraphPad Prism.

3. RESULTS

Glucose and glycogen levels

Hemolymph glucose concentration in males fed by 5S-5Y diet significantly depended on the developmental diet (ANOVA: $F_{1,25} = 11.62$, $p < 0.0001$), age (ANOVA: $F_{2,25} = 22.54$, $p < 0.0001$) and the interaction between diet and age (ANOVA: $F_{2,25} = 25.57$, $p < 0.0001$) (Table 2). Two 2-day-old males developed on Y-M diet displayed 3.5-fold lower hemolymph glucose concentration as compared to 5Y diet (Fig. 1A; H-S test: $p < 0.0001$). Moreover, we observed a 3.5-fold higher hemolymph glucose level in two-day-old males of Group I as compared to 15 and 40 days (Fig. 1A; H-S test: $p < 0.0001$).

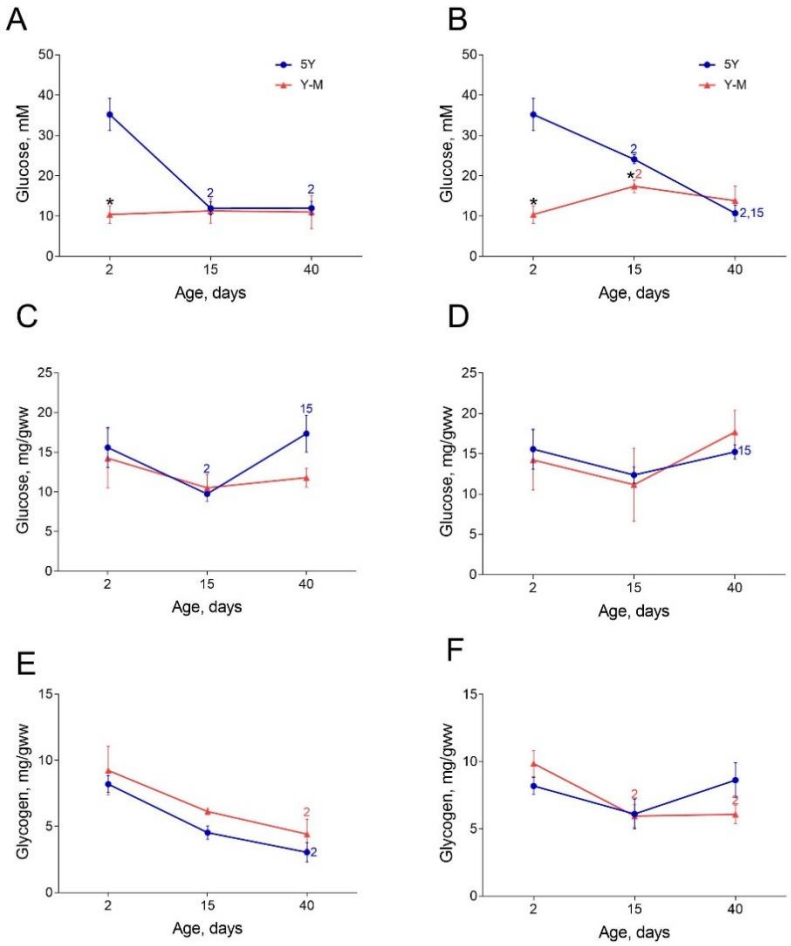

Figure 1. The level of carbohydrates in males of different ages developed on 5Y or yeast-molasses diet. Hemolymph glucose in males reared on 5S-5Y diet (A) and 20S-2Y diet (B), body glucose under 5S-5Y (C) and 20S-2Y (D), glycogen levels under 5S-5Y (E) and 20S-5Y (F). Results represent the mean ± SEM of 5-6 replicates. Group comparisons were performed using Holm-Sidak's test. Asterisk indicates a significant difference from 5S-5Y groups with $p < 0.05$. 2, 15 indicates a significant difference from means on the 2nd and 15th day, respectively

Similarly, hemolymph glucose concentration significantly depended on the developmental diet (ANOVA: $F_{1,23} = 40.22$, $p < 0.0001$), age (ANOVA: $F_{2,23} = 19.22$, $p < 0.0001$) and the interaction between diet and age (ANOVA: $F_{2,23} = 30.96$, $p < 0.0001$) in males fed by 20S-2Y (Table 2). Lower glucose concentration in hemolymph was found in males developed on Y-M diet on the 2nd and 15th day (Fig. 1A; H-S test: $p < 0.04$). Hemolymph glucose levels in males of Group III decreased in the following order: 2nd day > 15th day > 40th day (Fig. 1B; H-S test, $p < 0.0003$). Males of Group IV aged 15 days displayed significantly higher glucose concentration in hemolymph as compared to 2 days (Fig. 1B; H-S test, $p = 0.0220$).

Body glucose level was associated with age in males fed by either 5S-5Y diet (ANOVA: $F_{2,23} = 5.526$, $p = 0.0110$) or the 20S-2Y diet (ANOVA: $F_{2,23} = 4.308$, $p = 0.0258$) (Table 2). Lower body glucose content was found in 15-day-old males of Group I as compared to 2- and 40-day-old males (Fig. 1C; H-S test, $p < 0.05$). In males Group III body glucose content was higher on the 40th day as compared to the 15th day (Fig. 1D; H-S test, $p = 0.0222$).

Glycogen content was determined by age in males fed by either the 5S-5Y diet (ANOVA: $F_{2,21} = 11.11$, $p = 0.0005$) or the 20S-2Y diet (ANOVA: $F_{2,22} = 4.570$, $p = 0.0219$) (Table 2). A two-fold higher glycogen pool was observed in 2-day-old males fed by 5S-5Y diet as compared to 40-day-old regardless of developmental diet (Fig. 1E; H-S test, $p < 0.03$). Similarly, in 2-day-old males of Group

IV glycogen content was significantly higher as compared to 15- and 40-day-old males (Fig. 1F; H-S test, $p < 0.02$).

In female flies, glucose concentration in hemolymph was associated with developmental diet (ANOVA (5S-5Y): $F_{1,25} = 52.22$, $p < 0.0001$; ANOVA (20S-2Y): $F_{1,24} = 49.38$, $p < 0.0001$), age (ANOVA (5S-5Y): $F_{2,25} = 53.22$, $p < 0.0001$; ANOVA (20S-2Y): $F_{2,24} = 26.11$, $p < 0.0001$) and the interaction between diet and age (ANOVA (5S-5Y): $F_{2,25} = 30.94$, $p < 0.0001$; ANOVA (20S-2Y): $F_{2,24} = 17.09$, $p < 0.0001$) (Table 2). Consumption of the 5Y diet during development leads to lower glucose concentration in the hemolymph of females on the 2nd and 15th days as compared to the Y-M diet (Fig. 2A and B; H-S test, $p < 0.0001$). We observed 2.8-3-fold lower hemolymph glucose levels in 40-day-old females that developed on a 5Y diet as compared to 2- and 15-day-old females (Fig. 2A,B; H-S test, $p < 0.0001$).

Table 2. Two-way ANOVA to determine the impact of developmental diet and age on metabolic parameters in flies that were reared either on 5S-5Y diet, as well as on 20S-2Y diet

5S-5Y diet	Diet			Age			Diet and Age		
MALES	DF	*F* ratio	*P*	DF	*F* ratio	*P*	DF	*F* ratio	*P*
Hemolymph glucose	1, 25	31.34	<0.0001*	2, 25	22.54	<0.0001*	2, 25	25.57	<0.0001*
Body glucose	1, 23	2.489	0.1283	2, 23	5.526	0.0110*	2, 23	2.076	0.1483
Glycogen	1, 21	2.284	0.1456	2, 21	11.11	0.0005*	2, 21	0.0340	0.9666
Weight	1, 25	0.1779	0.6768	2, 25	1.386	0.2687	2, 25	0.5657	0.5751
Triglycerides	1, 25	7.575	0.0109*	2, 25	4.079	0.0293*	2, 25	2.940	0.0714
Lipids	1, 26	2.763	0.1141	2, 26	3.156	0.0593	2, 26	0.0511	0.9503
20S-2Y diet	Diet			Age			Diet and Age		
MALES	DF	*F* ratio	*P*	DF	*F* ratio	*P*	DF	*F* ratio	*P*
Hemolymph glucose	1, 23	40.22	<0.0001*	2, 23	19.22	<0.0001*	2, 23	30.96	<0.0001*
Body glucose	1, 23	0.0003	0.9858	2, 23	4.308	0.0258*	2, 23	0.9180	0.9503
Glycogen	1, 22	0.1854	0.6709	2, 22	4.570	0.0219*	2, 22	2.405	0.1136
Weight	1, 25	0.1746	0.6796	2, 25	1.714	0.2006	2, 25	0.2805	0.7577
Triglycerides	1, 24	0.4304	0.5180	2, 24	23.26	<0.0001*	2, 24	2.588	0.0960
Lipids	1, 24	0.0617	0.8059	2, 24	16.90	<0.0001*	2, 24	0.2339	0.7932
5S-5Y diet	Diet			Age			Diet and Age		
FEMALES	DF	*F* ratio	*P*	DF	*F* ratio	*P*	DF	*F* ratio	*P*
Hemolymph glucose	1, 25	52.25	<0.0001*	2, 25	53.22	<0.0001*	2, 25	30.94	<0.0001*
Body glucose	1, 23	0.0412	0.8409	2, 23	7.141	0.0039*	2, 23	0.7285	0.4937
Glycogen	1, 22	5.052	0.0349	2, 22	9.676	0.0010*	2, 22	2.030	0.1552
Weight	1, 27	0.6603	0.4236	2, 27	7.336	0.0029*	2, 27	0.9802	0.3882
Triglycerides	1, 24	13.52	0.0012*	2, 24	21.74	<0.0001*	2, 24	36.28	<0.0001*
Lipids	1, 24	2.654	0.1163	2, 24	3.852	0.0354*	2, 24	0.5623	0.5772
20S-2Y diet	Diet			Age			Diet and Age		
FEMALES	DF	*F* ratio	*P*	DF	*F* ratio	*P*	DF	*F* ratio	*P*
Hemolymph glucose	1, 24	49.38	<0.0001*	2, 24	26.11	<0.0001*	2, 24	17.09	<0.0001*
Body glucose	1, 23	0.9643	0.3363	2, 23	8.011	0.0023*	2, 23	0.7700	0.4746
Glycogen	1, 21	3.866	0.0626	2, 21	5.131	0.0153*	2, 21	3.169	0.626
Weight	1, 27	0.2844	0.5982	2, 27	16.70	<0.0001*	2, 27	0.3442	0.7119
Triglycerides	1, 23	19.48	0.0002*	2, 23	26.72	<0.0001*	2, 23	12.74	0.0002*
Lipids	1, 23	14.53	0.0009	2, 23	20.40	<0.0001*	2, 23	0.4159	0.6646

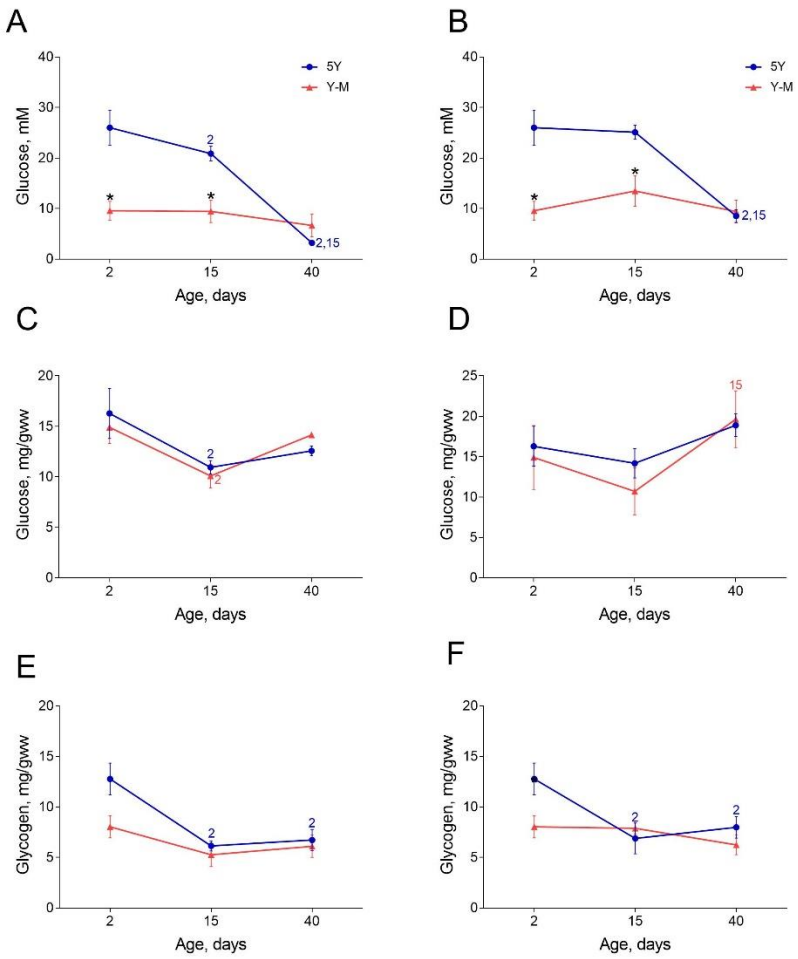

Figure 2. The levels of carbohydrates in females on the 2nd, 15th, and 40th day under consumption the 5Y or yeast-molasses diet during development. Hemolymph glucose in males reared on 5S-5Y diet (A) and 20S-2Y diet (B), body glucose under 5S-5Y (C) and 20S-2Y (D), glycogen levels under 5S-5Y (E) and 20S-5Y (F). Results represent the mean ± SEM of 5-6 replicates per group. Group comparisons were performed using Holm-Sidak's test. Asterisk indicates a significant difference from 5S-5Y groups with p < 0.05. 2, 15 indicates a significant difference from means on the 2nd and 15th day.

Body glucose level was determined by age in females fed by 5S-5Y diet (ANOVA: $F_{2,23} = 7.141$, $p = 0.0039$) and 20S-2Y diet (ANOVA: $F_{2,23} = 8.011$, $p = 0.0011$) (Table 2). We observed 28% higher body glucose levels in 2-day-old females fed by 5S-5Y as compared to 15th-day females regardless of developmental diet (Fig. 2C; H-S test, $p < 0.05$). In 40-day-old females of Group IV body glucose level was 46% higher, as compared to 15th-day females (Fig. 2D; H-S test, $p = 0.0027$).

Glycogen pool strongly depended on age in females fed by 5S-5Y diet (ANOVA: $F_{2,22} = 9.676$, $p = 0.0010$) and 20S-2Y diet (ANOVA: $F_{2,21} = 5.131$, $p = 0.0153$) (Table 2). We observed enhanced glycogen levels in 2-day-old females of Group I (47-52%) or Group III (37-47%) as compared to 15- and 40-day-old females (Fig. 2F; H-S test, $p < 0.04$).

Lipid storage level and body weight

Body weight in males was not affected either by developmental diet or age (Fig. 3A and B; Table 1). TAG content in males was dependent on both developmental diet (ANOVA (5S-5Y): $F_{1,25} = 7.575$, $p = 0.0109$) and age (ANOVA (5S-5Y): $F_{2,25} = 4.079$, $p = 0.0293$; ANOVA (20S-2Y): $F_{2,24} = 23.26$, $p < 0.0001$) (Table 2). In 15-day-old males of Group II the level of TAG was significantly higher as compared to males of Group I, as well as to 2- and 40-day-old males (Fig. 3C; H-S test, $p < 0.03$). We found a higher

level of TAG in 15-day-old males that consumed a high-sucrose diet as compared to 2-day-old males regardless of developmental diet (Fig. 3D; H-S test, $p < 0.006$).

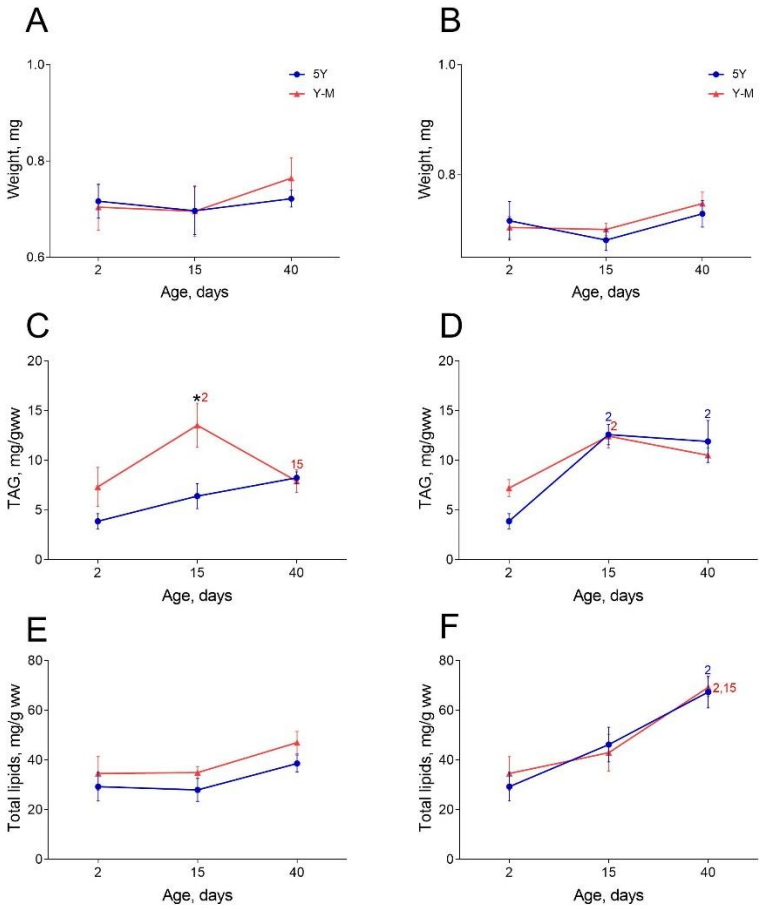

Figure 3. Body weight and lipid levels in males on the 2nd, 15th, and 40th day under consumption the 5Y or yeast-molasses diet during development. Body weight in males reared on 5S-5Y diet (A) and 20S-2Y diet (B), TAG level under 5S-5Y (C) and 20S-2Y (D), total lipid content under 5S-5Y (E) and 20S-5Y (F). Results represent the mean ± SEM of 5-6 replicates per group. Group comparisons were performed using Holm-Sidak's test. Asterisk indicates a significant difference from 5S-5Y groups with p < 0.05. 2, 15 indicates a significant difference from means on the 2nd and 15th day

In males who consumed 5S-5Y the level of total lipid amount was not associated with developmental diet and age (Fig. 3E). However, total lipid content in males that consumed the 20S-2Y diet was significantly affected by age (ANOVA: $F_{2,25} = 23.26$, $p < 0.0001$) (Table 2). We observed higher levels of total lipids in 40-day-old males as compared to 2-day-old males regardless of developmental diet (Fig. 3F; H-S test, $p < 0.002$).

In females, body weight was associated with age (ANOVA (5S-5Y): $F_{1,25} = 52.22$, $p < 0.0001$; ANOVA (20S-2Y): $F_{1,24} = 49.38$, $p < 0.0001$) (Table 2). We observed lower body weight in 15-day-old females regardless of the diet as compared to 2-day-old females (Fig. 4A and B; H-S test, $p < 0.007$). High-sugar diet led to increased body weight by 19% and 13% in females developed on the Y-M diet on the 2nd day, as compared 15th and 40th days, respectively (Fig. 4B; H-S test, $p < 0.005$).

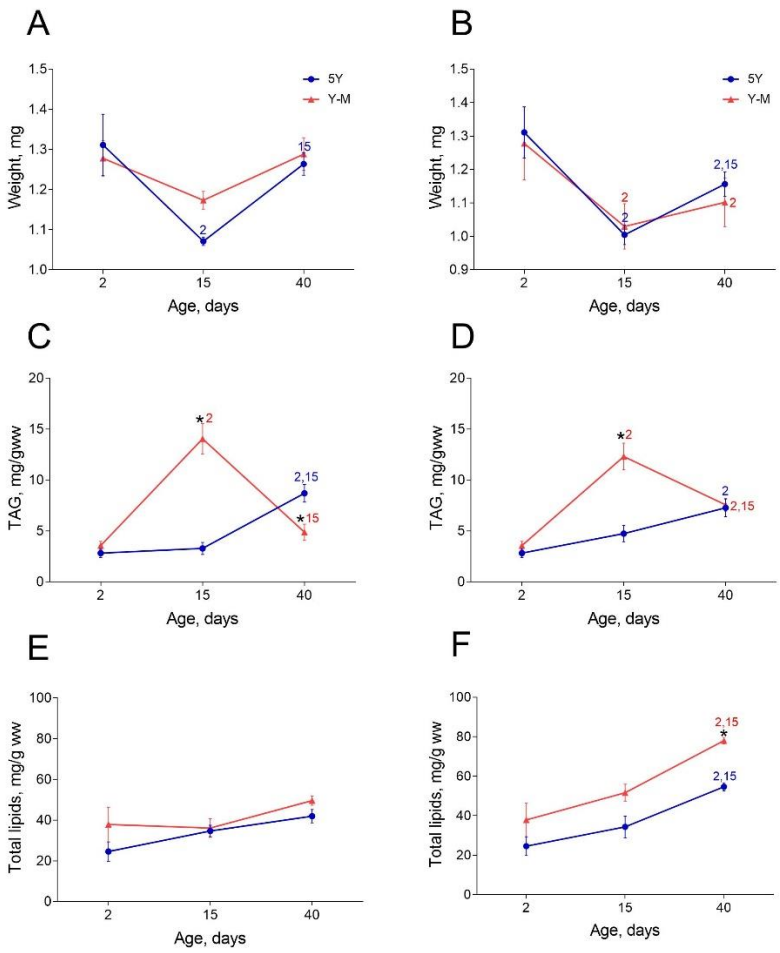

Figure 4. Body weight and lipid levels in females on the 2nd, 15th, and 40th day under consumption the 5Y or yeast-molasses diet during development. Body weight in males reared on 5S-5Y diet (A) and 20S-2Y diet (B), TAG level under 5S-5Y (C) and 20S-2Y (D), total lipid content under 5S-5Y (E) and 20S-5Y (F). Results represent the mean ± SEM of 5-6 replicates per group. Group comparisons were performed using Holm-Sidak's test. Asterisk indicates a significant difference from 5S-5Y groups with p < 0.05. 2, 15 indicates a significant difference from means on the 2nd and 15th day

TAG level in females was depended on developmental diet (ANOVA (5S-5Y): $F_{1,24} = 13.52$, $p = 0.0012$; ANOVA (20S-2Y): $F_{1,23} = 13.48$, $p = 0.0003$), age (ANOVA (5S-5Y): $F_{2,24} = 21.74$, $p < 0.0001$; ANOVA (20S-2Y): $F_{2,23} = 26.72$, $p < 0.0001$) and the interaction between diet and age (ANOVA (5S-5Y): $F_{2,24} = 36.28$, $p < 0.0001$; ANOVA (20S-2Y): $F_{2,23} = 12.74$, $p = 0.0002$) (Table 2). We observed a higher level of TAG in 15-days-old females developed on the Y-M diet which consumed either 5S-5Y (by 75%) or 20S-2Y (by 71%) diet as compared to 2-day-old females (Fig. 4C and D; H-S test, $p < 0.05$). A lower level of TAG by 65% was observed in 40-day-old females of Group II as compared to 15-day-old females (Fig. 4C; H-S test, $p < 0.0001$).

Total lipids content was affected by age (ANOVA (5S-5Y): $F_{2,24} = 3.852$, $p = 0.0354$; ANOVA (20S-2Y): $F_{2,23} = 20.40$, $p < 0.0001$) (Table 2). A high sucrose diet led to increased total lipids content in 40-day-old females as compared to 2- and 15-day-old females regardless of developmental diet (Fig. 4F; H-S test, $p < 0.05$). Consumption of the 20S-2Y diet for 40 days by females caused higher total lipids content as compared to females fed by 5S-5Y diet (Fig. 4F; H-S test, $p = 0.0310$).

4. DISCUSSION

Previous studies have shown that metabolite levels change with age in mice (Zhou et al., 2022) and humans (Yu et al., 2012). We were interested in whether developmental-diet-induced alterations of metabolite levels can be as large or larger than those associated with adult dietary regimens. In this study, we combined two types of developmental diet with two types of diet during the adult stage to examine the effects of dietary composition at different life stages and age. In particular, we were interested in whether the effect of age on metabolite levels would be dependent on the effects of a developmental diet. Different laboratories use different diets for breeding and maintaining fruit flies. The classic food for *Drosophila* maintained in our lab, standard brown food is a nutritional blend of quality molasses, enriched cornmeal, and inactivated yeast. Molasses is a byproduct of making sugar and contains more vitamins and minerals than other sweeteners but is still high in sugar. Molasses mostly consists of the disaccharide sucrose and smaller amounts of the monosaccharides fructose and glucose. As contrast, we also used a sucrose-free developmental diet that consist only of dry yeast during the larval stage.

It was previously demonstrated nutritional optima (P:C) 1:4 for lifespan, 1:2 for egg-to-adult viability, 1:1 for female body mass at adult eclosion and lifetime fecundity, 2:1 for larval developmental rate and 8:1 for egg production rate (Jang and Lee, 2018). Hence, we used two types of developmental diet to compare the life-history responses of adult *D. melanogaster* at different age.

Carbohydrate and Lipid Metabolism is Affected by Developmental Diet

Previous studies have repeatedly demonstrated the influence of developmental nutrition on *Drosophila* lifespan, metabolism and overall performance (Aguila et al., 2007; Musselman et al., 2011; May et al., 2015; Strilbytska et al., 2022). Consistent with these previous results we found that nutrition during the larval stage influences the level of circulating glucose and TAG levels of both male and female flies. Moreover, this effect was observed when flies were fed by standard diet (5S-5Y) or by high-sugar diet (20S-2Y) after eclosion. Flies that developed on the Y-M diet, displayed lower hemolymph glucose concentration as compared to the 5Y diet. However, the Y-M developmental diet led to higher TAG content as compared to the 5Y diet. Nevertheless, the effect of developmental diet on the concentration of hemolymph glucose and TAG was observed only in 2- and 15-day-old flies. Hence, the consumption of a fixed diet during the long period of the adult stage can equalize the impact of a developmental diet. The lasting effects of developmental diet were established previously (Stefana et al., 2017; Jang and Lee, 2018; Henry et al., 2019; Shukla and Kolthur-Seetharam, 2022; Zúñiga-Hernández et al., 2023). Restriction of dietary yeast induces long-term changes in adult triglyceride storage that are strongly associated with extended lifespan (Stefana et al., 2017). Moreover, switching adults to a high-yeast diet did not influence the longevity effects of a low-yeast developmental diet (Stefana et al., 2017). Sirtuin 6 was demonstrated to be involved in nutrient-dependent larval developmental kinetics in *Drosophila* (Shukla and Kolthur-Seetharam, 2022). Sirt6 maintains glucose and TAG homeostasis in response to changes in developmental nutrient conditions (Shukla and Kolthur-Seetharam, 2022). Regulation of developmental pathways such as ecdysone signaling is realized via TOR/insulin signaling. Early-life nutrition imprints epigenetic and transcriptional changes in genes associated with excitability and connectivity in Kenyon cells (Zúñiga-Hernández et al., 2023).

Metabolites Affected by Age

We also studied the effect of age on metabolic parameters. Our data revealed that metabolite content in both males and females was strongly associated with age. All investigated metabolites were significantly affected by age. Hemolymph glucose concentration was higher in young flies of both sexes, developed on a 5Y diet, compared to adult and old flies. Body glucose levels were higher in old flies, regardless of developmental diet. Glycogen content was higher in young male and

female flies, developed on a 5Y diet, compared to adult and old flies. Indeed, it was previously shown that the reserves of body glycogen fall as *Drosophila* ages (Driver and Lamb, 1980). Adult flies showed the highest level of TAG as compared to young or old ones. Total lipids content was maximized in old flies regardless of developmental diet. It was recently indicated that nutrient stores in the form of TAG, within lipid droplets are important to maintain organismal energy homeostasis. In fact, as reported by Driver and Lamb (1980) it is falling the rate of metabolism with age. Lower uptake of nutrients from the gut or a greater excretion of food substances may contribute to age-related reduction of metabolic rate at the cellular level. Higher levels of hemolymph and body glucose, TAG and total lipid may be caused by alterations in feeding behavior during adult life. Indeed, it was demonstrated that as *Drosophila* ages, the amount of food eaten per day increases (Driver and Lamb, 1980). Changes in feeding behavior may lead to age-related changes in insulin and insulin-growth-factor-like signaling (IIS) (Tanabe et al., 2017; Semaniuk et al., 2021a, b). Aging significantly contributes to the decrease in dilp3 relative expression in a dFOXO-dependent manner (Broughton et al., 2008).

Aging is associated with changes in genetic, transcriptomic and translational levels that, in turn, influence on metabolism. Expression pattern of nearly 23% of genes was shown to change as *Drosophila* age (Pletcher et al., 2002). These genes are involved in the regulation of various cellular processes, including metabolism. Genes linked with oxidative phosphorylation, TCA cycle, ATP production are downregulated during *Drosophila* aging. Mitochondrial respiration has been shown to be altered with age (Ferguson et al., 2005). Reactive oxygen species (ROS) are among the main mechanisms that mediate the effect of nutrient consumption on the aging process. Dietary carbohydrates were shown to have a significant impact on oxidative metabolism in *D. melanogaster* (Strilbytska et al., 2022). However, it was also shown that tissue-specific consequences of aging on metabolism (Girardot et al., 2006). It was demonstrated shift in microbiota composition in old flies that impair intestinal function (Clark et al., 2015). Moreover, age-related dysbiosis led to a shift in excretory function (Clark et al., 2015). We suggested that all these events may have a significant impact on metabolic functions and aging.

5. CONCLUSION

In this study, we have been able to follow the dynamics of the carbohydrate and lipid metabolism throughout the lifespan of *Drosophila* that depend on nutrition during development. In conclusion, we found that the metabolite levels in flies developed on 5Y diet differ from the Y-M diet only at a young age, which led us to suggest that the developmental diet did not influence fly response to aging. However, we found that the combination of developmental and adult environments can also determine metabolic outcomes, not one or the other.

Conflict of interest

The authors declare that they have no conflict of interest.

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Надія Стефанишин, Ольга Стрільбицька, Ігор Юркевич, Надія Бурдилюк, Олег Лущак. Дієта під час розвитку частково детермінує вікові зміни метаболізму *Drosophila*. *Журнал Прикарпатського університету імені Василя Стефаника*, 10 (2023), 7-19.

Старіння і метаболізм нерозривно пов'язані між собою, і обидва процеси мають вирішальне значення для довголіття. Зміни в енергетичному метаболізмі відбуваються під час нормального старіння і можуть бути частково покращені шляхом зміни способу життя. Макроелементи значно впливають на ознаки життєдіяльності, такі як вразливість до хвороб, розмноження, тривалість життя та стресостійкість. Ми дослідили вікові зміни метаболізму у плодових мушок *Drosophila melanogaster*, яких годували різними дієтами на стадії розвитку. Дорослі мухи були переведені на інші два види дієт. Протестовано двох-, 15- або 40-денних мух з кожної експериментальної групи. Ми спостерігали значні зміни в обміні вуглеводів та ліпідів, які пов'язані з раціоном під час розвитку та з віком. Харчовий раціон під час розвитку впливає на рівень глюкози та тригліцеридів у гемолімфі молодих та дорослих мух. Отже, споживання фіксованого раціону протягом тривалого періоду дорослої стадії може нівелювати вплив дієти розвитку. Вищий рівень глюкози в гемолімфі спостерігали у мух, яких годували середовищем, що містило 5% дріжджів, порівняно з дріжджово-мелясною мелясою під час розвитку. Однак, вищі рівні ТАГ та ліпідів були зумовлені споживанням дріжджово-мелясного середовища під час розвитку. З віком запаси вуглеводів зменшуються з одночасним збільшенням запасу ліпідів. Вплив харчування та віку на розвиток та обмін речовин не пов'язаний зі статтю або харчуванням протягом дорослого життя. Потенційними механізмами, залученими у регуляції розвитку за різних умов доступності харчових компонентів, можна вважати інсуліновий сигнальний шлях, а також мішень дії рапаміцину (TOR). Наші дані чітко показують зв'язок між умовами розвитку та змінами метаболізму зі старінням у дрозофіли.

Ключові слова: старіння; метаболізм; вуглеводи; плодова мушка; розвиток.