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High amylose starch consumption induces obesity in *Drosophila melanogaster* and metformin partially prevents accumulation of storage lipids and shortens lifespan of the insects

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ABSTRACT

There are very few studies that have directly analyzed the effects of dietary intake of slowly digestible starches on metabolic parameters of animals. The present study examined the effects of slowly digestible starch with high amylose content (referred also as amylose starch) either alone, or in combination with metformin on the development, lifespan, and levels of glucose and storage lipids in the fruit fly *Drosophila melanogaster*. Consumption of amylose starch in concentrations 0.25–10% did not affect *D. melanogaster* development, whereas 20% starch delayed pupation and reduced the number of larvae that reached the pupal stage. Starch levels in larval food, but not in adult food, determined levels of triacylglycerides in eight-day-old adult flies. Rearing on diet with 20% starch led to shorter lifespan and a higher content of triacylglycerides in the bodies of adult flies as compared with the same parameters in flies fed on 4% starch diet. Food supplementation with 10 mM metformin partly attenuated the negative effects of high starch concentrations on larval pupation and decreased triacylglyceride levels in adult flies fed on 20% starch. Long-term consumption of diets supplemented with metformin and starch decreased lifespan of the insects, compared with the diet supplemented with starch only. The data show that in *Drosophila* high starch consumption may induce a fat fly phenotype and metformin may partially prevent it.

1. Introduction

Excessive intake of food, particularly carbohydrates, promotes obesity and increases the risk of many chronic diseases, including type 2 diabetes, cardiovascular and neurodegenerative diseases (Kahn and Flier, 2000; Baker and Thummel, 2007; Hong and Park, 2010; Kanasaki and Koya, 2011). Therefore, low carbohydrate diets have been proposed as interventions to reduce the risk of complications associated with metabolic disorders (Samaha et al., 2003; Giugliano et al., 2008). The nutritional properties of carbohydrates depend not only on their quantity, but also on the rate and extent of their digestion and absorption. For example, starches with high amylose content are more resistant to digestion than starches with higher amylopectin levels (Englyst et al., 1992; Topping and Clifton, 2001; Rosin et al., 2002; Tapsell, 2004; Aller et al., 2011). Since starch is the most abundant form of carbohydrate in the human diet, its replacement with so-called resistant and slowly digestible starches have been actively studied in recent years as a new approach to ameliorate the possible risks of high carbohydrate diets. Based on clinical and animal research, starch with higher amylose content (referred as an amylose starch) has been proposed to be the most potentially beneficial starch fraction for human health (Englyst et al., 1992; Topping and Clifton, 2001; Zhang and Hamaker, 2009; Rosin et al., 2002; Aller et al., 2011). However, despite some benefits of resistant or slowly digestible starches, the detailed dose-dependent effects of this carbohydrate fraction on health-promoting mechanisms have not yet been established.

Recent studies showed that model organisms like *Drosophila melanogaster* can be used efficiently in nutritional studies to explore new regulatory mechanisms associated with metabolism (Baker and Thummel, 2007; Hong and Park, 2010; Slack et al., 2012; Rajan and Perrimon, 2013; Lushchak et al., 2014; Smith et al., 2014; Rovenko et al., 2015a). Studies with *D. melanogaster* have shown the conservation of regulatory mechanisms involved in metabolic homeostasis between mammals and insects, in particular pathways associated with fat metabolism, adipocyte development, and insulin signaling (Baker and Thummel, 2007; Smith et al., 2014). We previously reported the effects of diets enriched with sugars such as glucose, fructose and sucrose on lifespan and metabolic parameters of *D. melanogaster* (Rovenko et al.,

Abbreviations: PT_{50}, median pupariation time; TAG, triacylglycerides

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2015a, 2015b). Our studies (Rovenko et al., 2015a, 2015b) and from others (Musselman et al., 2011; Slack et al., 2012) found that mono- and disaccharides at high concentrations contributed to metabolic disorders in the fly body, and as such, we suggested that similar effects might occur with excessive consumption of starch. Therefore, in this work we studied the effects of diets supplemented with amylose starch in different concentrations on the development, some metabolic indices, and lifespan of *D. melanogaster*. Receiving initial results that indicated development of a hyperlipidemic phenotype when flies were fed on high starch diets, we then tested whether the drug metformin could modulate effects of starch-containing diets. Metformin (*N*,*N*-dimethylimidodicarbonimidic diamide) is widely used as a treatment for type 2 diabetes because of its known hypoglycemic and hypolipidemic effects (Bailey and Turner, 1996; Malin and Kashyap, 2014; Fantus, 2015; Adeyemo et al., 2015).

2. Material and methods

2.1. Reagents

Potassium phosphate monobasic, phosphate buffered saline, and Tween 20 were purchased from Sigma-Aldrich Corporation (USA). Diagnostic kits for the determination of glucose and triacylglycerides were obtained from P.Z. Cormay S.A. (Łomianki, Poland). Corn starch with high amylose content (about 47% according to information from the producer Khimlaborreaktiv), metformin (1,1-dimethylbiguanide hydrochloride) and all other reagents were of the analytical grade from local suppliers (Ukraine).

2.2. Drosophila melanogaster stock and media

The *D. melanogaster* strain w^{1118} was obtained from Bloomington Stock Center (Bloomington, Indiana, USA). Stock flies and larvae were reared on a standard yeast-corn-molasses food with 12 hour illumination at 25 ± 1 °C and relative humidity of 55–60%. Nipagin (methyl-*p*-hydroxybenzoate) at a concentration of 0.18% (v/v) was added to the medium to inhibit mold growth (Lozinsky et al., 2013). Experimental media contained 2% yeast (w/v), 0.18% nipagin (v/v), 1% agar (w/v) and amylose starch in a range of concentrations from 0.25 to 20% (w/v). In another series of studies, experimental media were also supplemented with metformin (0.1–50 mM).

2.3. Pupariation

Eggs were collected 3–4 h after laying and transferred into bottles containing food with different concentrations of starch (about 150 eggs per bottle containing 15 mL of food). Eggs were hatched and larvae were developed until pupae in these bottles. The number of puparia formed was recorded every 24 h over 5 days. Median pupariation time was calculated accordingly to Olcott et al. (2010) as the time at which 50% of total larvae had pupariated (PT_{50}). Percentage of eggs that pupated was calculated as the number of pupated larvae divided by the total number the eggs initially placed in the bottles.

2.4. Effect of experimental media on larval behavior (pupation height)

Changes in larval behavior in response to the experimental exposures were assessed as pupation height preference. Before pupation, larvae climb up the walls of the bottles, attach themselves and then pupate. After 4–6 days when all larvae had pupated, pupation heights were measured as the distance from the food surface (pupation on the food surface was scored as zero). The pupation height was measured millimeters (mm) as described previously (Singh and Pandey, 1991).

2.5. Food intake by larvae

Food intake was measured as described previously (Lushchak et al., 2011). Briefly, groups of 15 third instar larvae were reared on starch media and then placed for 20 min on the same food but containing 0.5% dye FD & C Blue No. 1 (Brilliant Blue FCF) poured in Petri dishes. After feeding, each group of larvae was washed to remove external dye adhering to the larvae and then immediately frozen in liquid nitrogen. For analysis, the groups of larvae were homogenized in 50 mM potassium phosphate (KPi) buffer (pH 7.0) in a ratio 1:100 (milligrams of larvae: microliters of buffer) and centrifuged at room temperature at 13,500*g* for 15 min. Supernatants were transferred to new tubes and mixed with KPi buffer in a ratio 1:1 (microliters of supernatant: microliters of buffer). Absorbance of the dye was measured at 629 nm and compared against a calibration curve built with different concentrations of dye. Optical densities of the supernatant samples from larvae that consumed corresponding diets without the dye were used as blanks. The total amounts of food consumed and carbohydrate eaten were calculated based on the dye absorbance. The number of calories consumed was calculated as described by Southgate and Durnin (1970).

2.6. Glucose and triacylglyceride assays

Experimental flies throughout larval development and imago stages (eight-day-old) were fed by the food containing amylose starch in a range of concentrations from 0.25 to 20%, 2% yeast, 1% of agar–agar, and 0.18% nipagin. In another series of studies, experimental groups of flies were fed by the amylose starch food supplemented with metformin (10 mM). Eight-day-old flies were mildly anesthetized with carbon dioxide gas, separated by sexes, and then quickly frozen in liquid nitrogen for further metabolic analysis. Glucose levels in the body were measured using a diagnostic kit Liquick Cor-GLUCOSE (PZ Cormay S.A., Poland) according to the protocol described in detail previously (Rovenko et al., 2014). To determine triacylglyceride levels, 6–10 preweighed flies were homogenized in chilled 10 mM phosphate-buffered saline with 0.05% Tween 20 (PBST buffer) (pH 7.4) in a ratio 1:50 (milligram of flies: microliters of buffer) at 4 °C. Homogenates were heated at 70 °C for 10 min followed by cooling to 4 °C (Tennessen et al., 2014). To precipitate denatured proteins, supernatants were centrifuged (3000*g*, 15 min, 21 °C). Final supernatants were used for assay of body triacylglycerides (TAG) by a diagnostic kit, Liquick Cor-TG (PZ Cormay S.A., Poland), following kit guidelines. TAG solutions ranging from 3 to 30 μg/mL were used to create a standard curve. Triacylglyceride levels in fly bodies were expressed as micrograms per milligram wet weight of flies (μg/mg ww).

2.7. Lifespan assay

Experimental flies were raised at standard densities of 150 eggs per bottle on 15 mL of yeast–molasses medium. Newborn flies were transferred to fresh medium and allowed to mate for 2 days. After separation by sexes, five day-old flies were placed into mortality cages with 5 mL of the experimental food. Food was changed every second day, and deaths were counted. To minimize any density effects on mortality, two vials within cohorts were merged when the density of flies reached 50% of the initial value (Lushchak et al., 2014). Three independent trials with about 100 flies per diet and sex were performed. The lifespan curves for each diet and sex represent cumulative survival for about 300 flies.

2.8. Statistical analysis

Data are presented as means ± S.E.M., with n = 4 independent replicates in most cases (as detailed in figure legends). One-way ANOVA was applied to all data followed by multiple comparisons, using SNK test of the Mynova program (version 1.3). The correlation

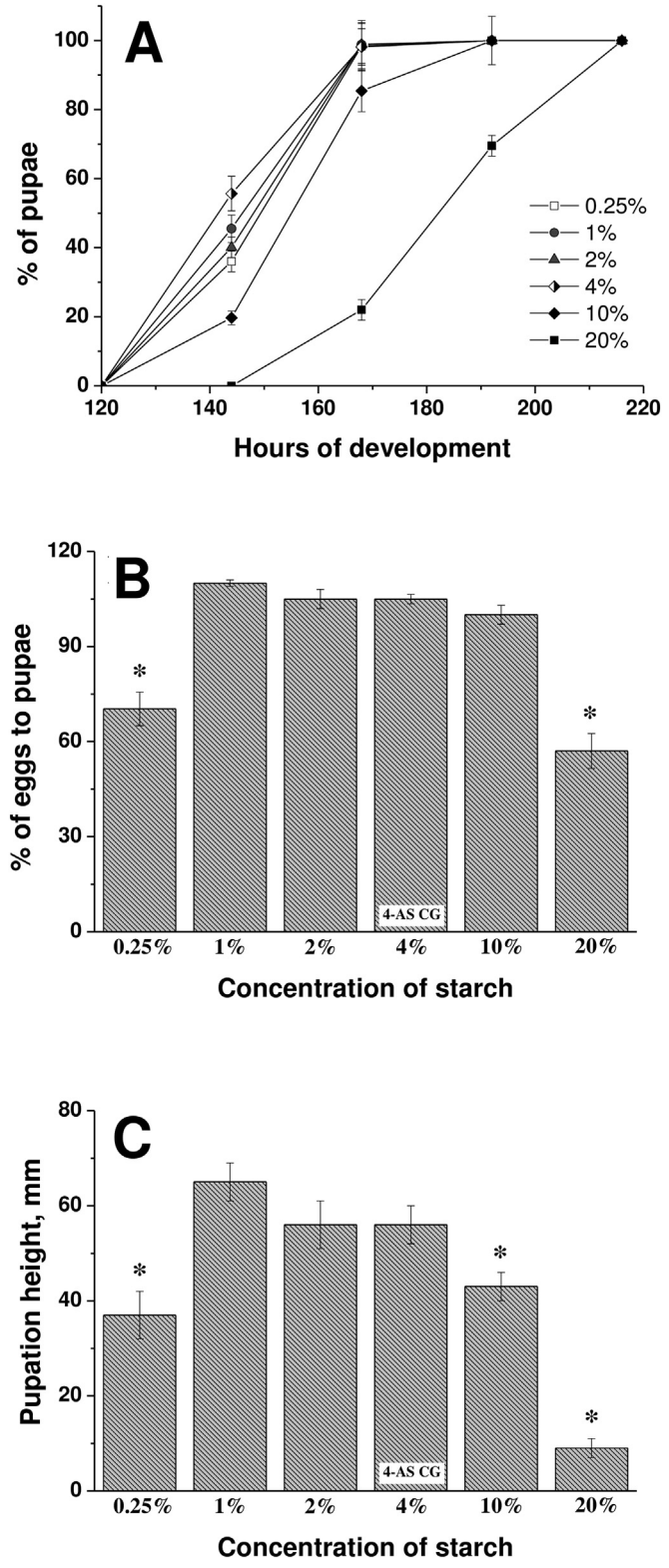

Fig. 1. Developmental (pupariation) curves (A), percent of pupated larvae (B) and pupation height (C) of *D. melanogaster* w^{1118} grown on media containing amylose starch in different concentrations. Data are presented as means ± S.E.M. In each assay cohorts with 600 individuals were used with n = 4 replicates. Diet with 4% amylose starch was taken as the comparison group (4-AS CG). *Significantly different from the 4-AS CG with $P < 0.05$.

coefficient and significance of correlation also were calculated using of the Mynova program. Differences in survival between cohorts were assessed by a log-rank test (Peto and Peto, 1972), using JMP 9.0 statistical software (SAS Institute). Median survival time was calculated from the average survival in cohorts.

3. Results and discussion

Drosophila melanogaster was found to be well-adapted to starch consumption (Fujimoto et al., 1999). In the current study, we used fruit flies to investigate some physiological effects of a corn starch with 47% amylose content. This type of starch also referred as a slowly digestible high amylose starch (Sandstedt et al., 1962), because amylose content in native corn starch is about 22% (Singh and Singh, 2003). Here we investigated effects of an amylose starch diet at different concentrations and in combination with metformin.

3.1. High amylose starch diet retards development of D. melanogaster

First, we evaluated the dynamics of fly development on diets containing amylose starch at different concentrations (Fig. 1). Six concentrations ranging from 0.25% to 20% w/v amylose starch were used. Among the preferred diets, the development of insects on 4% starch occurred rapidly. Besides, in many cases standard *Drosophila* diet composed of 4–5% carbohydrate. Therefore, the experimental medium supplemented with 4% amylose starch was selected as the comparison group (referred also as 4-AS group) and all subsequent parameters were compared to flies fed with 4% amylose starch. Larvae eating diets containing 0.25–2% amylose starch showed pupation patterns that were virtually the same as the 4-AS group, whereas consumption of diets with higher starch concentrations (10–20%) resulted in significantly prolonged development times (Fig. 1A). Median pupariation time (PT_{50}) (calculated from developmental curves in Fig. 1A) on 0.25%; 1%; 2%; 4%; 10% and 20% starch were 147 ± 4; 145 ± 3; 146 ± 4; 144 ± 3; 154 ± 6, and 175 ± 5 h, respectively. The values for PT_{50} differed significantly ($P < 0.05$) from the 4-AS group only in the two last cases. The influence of distinct carbohydrates on the development of fruit flies has previously been tested (Musselman et al., 2011; Rovenko et al., 2015a, 2015b; Lushchak et al., 2014) and delays in developmental time to pupariation of larvae reared on diets with high concentrations of glucose, fructose or sucrose were found (Musselman et al., 2011; Rovenko et al., 2015b). Our results here also show this trend of the negative influence of high concentrations of carbohydrates on fly development. However, in previous studies mono- and disaccharides were tested, whereas in the current study we tested the effects of polysaccharide starch. At least two points should be noted: (1) before absorption in the intestine, starch has to be digested, and (2) high starch concentrations in agar may be mechanically less acceptable as food for flies. As a consequence, the developmental delay noted for flies fed on 10% and 20% starch might be related to either or both of these factors – high concentrations of carbohydrates and perhaps also the physical properties of the substrate.

The larvae reared on low (0.25%) and high (20%) carbohydrate diets also had a lower population densities, because a significantly lower percentage of individual animals, just 67 and 54%, successfully developed from egg to pupa, respectively (Fig. 1B). Similar findings were reported by Johnson and Carder (2012). The authors showed that pupation height was proportionally influenced by larval density (the lower the larval density, the lower the pupation height). Our study shows similar results (Fig. 1B, C). Pupation height differed between the 4-AS group and cohorts fed on 0.25%, 10% or 20% starch that showed heights that were significantly reduced by 34%, 23% and 84%, respectively, compared with 4-AS group (Fig. 1C). Another study showed that larvae that developed more slowly also pupated closer to the food surface than larvae that developed normally (Casares and Carracedo, 1987). This phenomenon could also be one of the explanations for our observed effect. Since pupation height is an indicator of the functional state of the larvae (Sokolowski, 1985), we hypothesize that starch at low concentrations did not provide enough resources for the normal

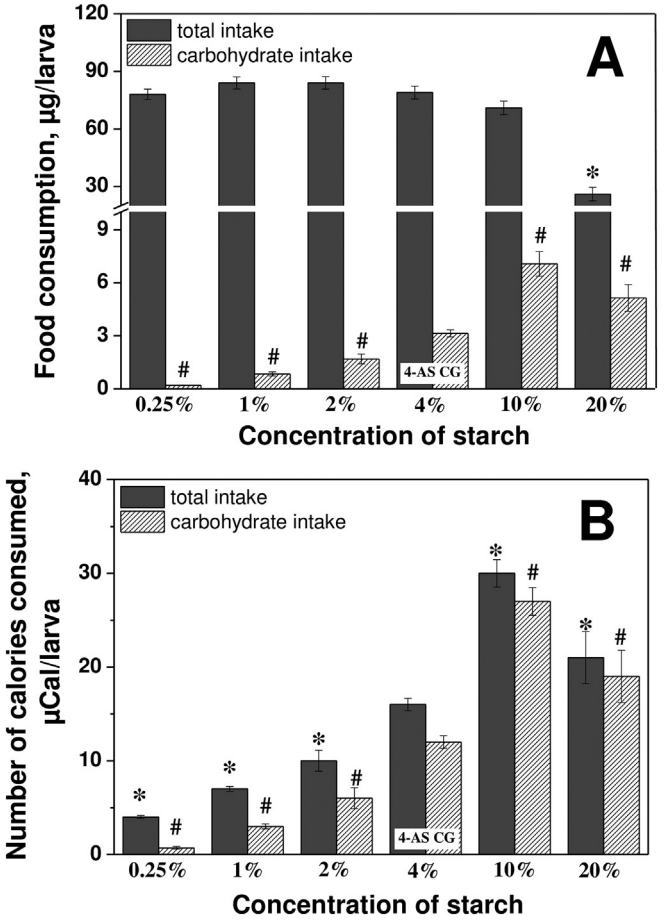

Fig. 2. Amount of food consumed by *D. melanogaster* w^{1118} larvae (A) and calories consumed (B) on different amylose starch diets. Data are presented as means ± S.E.M. In each assay groups with 120 larvae were used, with n = 4 replicates. Significantly different from the 4% amylose starch comparison group (4-AS CG) with respect to *total intake or #carbohydrate intake, P < 0.05.

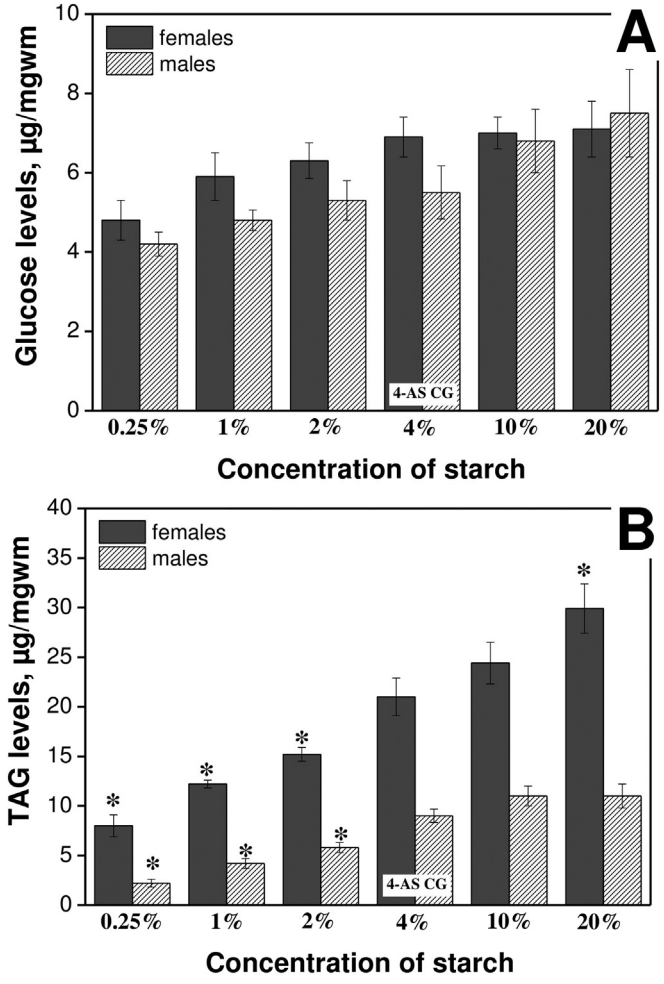

Fig. 3. The levels of glucose (A) and triacylglycerides (TAG) (B) in the bodies of eight-day-old *D. melanogaster* w^{1118} grown on media containing amylose starch at different concentrations. Data are presented as means ± S.E.M. (n = 6). *Significantly different from the respectively 4% amylose starch comparison group (4-AS CG), P < 0.05.

development of the insects. Negative effects due to high concentrations of starch in the diet could result from metabolic peculiarities in larvae which affected hormonal regulation of their development. We also consider that the delay in fly development could be caused by excessive solidification of the culture medium, making the food less accessible for larvae.

To distinguish between these two possible causes for delayed pupation (metabolic peculiarities and/or solidification of the culture medium) we evaluated food consumption by larvae reared on the experimental media as assessed by the intake of Brilliant Blue FCF dye. The volume of food intake on 20% starch was 70% lower as compared with the control diet (Fig. 2A). However, calculations showed that the larvae consumed about 2-fold more carbohydrate when fed on 10% and 20% starch diets, respectively, than on the 4% carbohydrate diet (Fig. 2A). Furthermore, caloric intake was 2.2- and 1.6-fold higher on diets with 10% and 20% starch, respectively, compared with 4-AS group (Fig. 2B). Thus, delayed development of larvae on high starch diets was not related to a lack of carbohydrate nutrients caused by difficulty in accessing food.

3.2. Amylose starch concentration in larval food determines TAG levels in adult flies

Previous studies analyzing the influence of monosaccharide or sucrose diets on larvae body composition showed that hyperglycemia and accumulation of lipid reserves might be a reason for the developmental delay of *D. melanogaster* maintained on high carbohydrate diets (Musselman et al., 2011; Rovenko et al., 2015a, 2015b; Lushchak et al., 2014). In our study, the negative effects of starch on fly development might also be associated with such metabolic perturbations. We examined the levels of glucose and TAG in eight-day-old imago fruit flies that consumed the different amylose starch diets (Fig. 3). Larvae were grown on different amylose starch diets (0.25–20%) and newborn flies were transferred to fresh experimental media with the same amylose content as they had as larvae. After eight days the flies were collected and immediately frozen for metabolic analysis. The data showed that glucose levels in the bodies of both fly sexes were not significantly different at any starch concentrations compared with 4-AS group, although a slight trend of increasing glucose was seen at higher starch concentrations (not significant) (Fig. 3A). This may be due to the fact that in flies, similar to mammals (Aller et al., 2011), amylose starch is digested very slowly, providing sustained glucose release with low initial glycemia as compared to more rapidly digestible starch. At the same time, fly consumption of the food with higher starch concentrations resulted in much higher TAG accumulation, especially in females (Fig. 3B). For example, TAG levels in females of the highest carbohydrate diet group (20% starch) were about 1.4-fold higher than in cohorts fed on 4% carbohydrate and about 4-fold higher than flies receiving the lowest (0.25%) starch diet (Fig. 3B). The levels of TAG in both males and females significantly correlated with the amount of carbohydrate and/or calories consumed. The correlation coefficient between TAG levels in adult males and larval calories consumed was

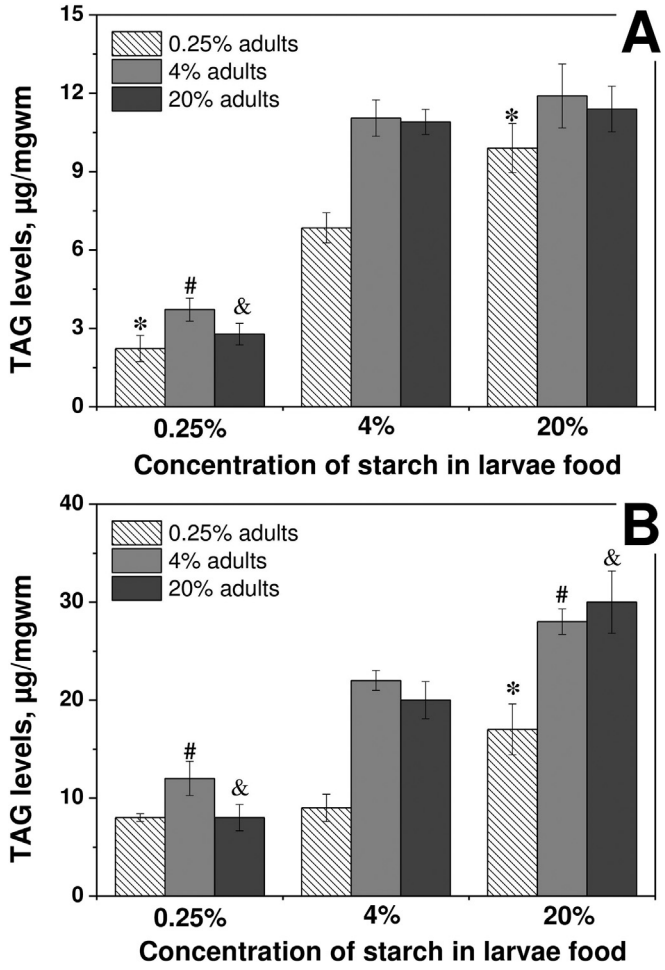

Fig. 4. Dependence of TAG levels in the bodies of eight-day-old male (A) and female (B) flies on nutrition in the larval stage. Larvae were fed different amylose starch diets: 0.25%, 4% or 20%. Each group flies infants divided into three parts and each part was transferred to new experimental medium with 025%, 4% or 20% starch (refer to the legend). Overall, then, each of the three cohorts was placed in three different starch diets: 0.25% larvae transferred to 0.25, 4, or 20% as adults; 4% larvae transferred to 0.25, 4, or 20% as adults; and 20% larvae transferred to 0.25, 4, or 20% as adults. Data are presented as means ± S.E.M. (n = 4). Significantly different from the control respectively comparison group: *4/0.25 (larvae/adults); #4/4; &4/20, P < 0.05.

0.97 ± 0.12 and in female it was 0.95 ± 0.16. This indicates that despite low digestion, prolonged consumption of slowly digestible starch led to excessive caloric intake resulting, in turn, in TAG accumulation. Our results regarding the effects of starch diets are in very good agreement with previous studies with *Drosophila* on the effects of other carbohydrates on TAG accumulation (Musselman et al., 2011; Rovenko et al., 2015b).

Interestingly, TAG level in adult flies was related to the starch content in the food at the larval stage (Fig. 4). In this experiment larvae were grown on different amylose starch diets (0.25%, 4% and 20%). Newly eclosed adults of each group were then divided into three subgroups and each subgroup was transferred to new experimental media either 0.25%, 4%, or 20% starch. Overall, then, each of the three cohorts was placed in three different starch diets: 0.25% larvae transferred to 0.25, 4, or 20% as adults; 4% larvae transferred to 0.25, 4, or 20% as adults; and 20% larvae transferred to 0.25, 4, or 20% as adults. The level of TAG in males reared on low starch food after eclosion, was proportional to the amount of dietary carbohydrate they ate during larval stage (Fig. 4A). Particularly, eight-day-old males of group 0.25/0.25 (developed on the medium with 0.25% starch and reared on the same medium after eclosion) had 3-fold lower TAG content than those of group 4/0.25. On the other hand, TAG content in males of group 20/0.25 was 1.4-fold higher than in males of group 4/0.25. Similar effect was observed also in females, which consumed low starch medium during larval stage (Fig. 4A). Female groups 0.25/0.25 and 4/0.25 did not differ significantly, while TAG content of female group 20/0.25 was 1.9-fold higher as compared to the group 4/0.25. There was no difference in TAG content between males, which consumed high starch and medium starch food during larval stage, while this difference was observed in females (Fig. 4A). Groups 0.25/4 and 0.25/20 of both sexes had significantly lower TAG content than respective groups 4/4 and 4/20. Hence, amount of dietary carbohydrate during larval stage defines TAG content in adult insects, whereas diet of adults did not influence this parameter. This effect is more pronounced in females (Fig. 4B). Our findings are consistent with an influence of nutrition in early life on metabolism and physiological processes programming in adult individuals. Recently, many researchers have shown that early life events can contribute substantially to the probability of an obese phenotype in adult and aged individuals (Vaiserman, 2011; Spencer, 2012; Aguila et al., 2013).

3.3. Metformin partially prevents accumulation of storage lipids and shortens lifespan of the insects

Metformin is known to decrease blood glucose levels (Klip and Leiter, 1990; Subodh et al., 1994; Cusi et al., 1996; Gonzalez-Angulo and Meric-Bernstam, 2010; Phielix et al., 2011) and promote fatty acid catabolism to limit lipid accumulation (Collier et al., 2006; Slack et al., 2012; Malin and Kashyap, 2014; Shirazi et al., 2014). Therefore, we suggested that metformin could prevent negative effects of high-starch diets on fruit flies. To test this, we evaluated the effects of metformin on fly development and TAG content in flies on starch diets.

Fig. 5A shows the dose-dependent effects of metformin on development *D. melanogaster* larvae on the 4% amylose starch diet in the presence of different concentrations of metformin. The experimental medium without metformin was selected comparison group (referred also as control). Diets with 0.1–25 mM metformin had no significant effect on larval pupariation time as compared to diets without metformin. However, feeding flies with food supplemented at the highest metformin concentration (50 mM) prolonged half-pupariation time by 17%. Previous studies with *Drosophila* reported that increasing the concentration of metformin on a sucrose diet above 10 mM resulted in a dose-dependent lifespan decrease (Slack et al., 2012). Therefore, an experimental medium with 10 mM metformin, selected as being a non-toxic concentration for insects was selected for a subsequent study. Metformin at 10 mM significantly prolonged the developmental time of fruit flies on the low starch diet (0.25%) by 8% and accelerated pupariation on the high-starch diet (20%) by 11% (Fig. 5B). Hence, metformin at a concentration of 10 mM partially alleviated the negative effect of high starch concentrations on fly development.

In the next series of experiments, we measured the levels of TAG in adult flies reared on different starch diets with or without 10 mM metformin. In these cases, the larvae were grown on different amylose starch diets (0.25–20%) supplemented or not metformin and newborn flies were transferred to fresh experimental media with the same composition as they had as larvae. The level of stored lipids in flies fed on the 0.25% starch diet was lower than that in flies fed on the 4% starch in both sexes for all conditions used (Fig. 6). For flies raised on the 20% starch diet, it was found that TAG levels were not significantly different in males compared with the 4% starch diet (Fig. 6A) but were 1.4-fold higher in females (Fig. 6B) on the 20% starch diet. However, in males and females maintained on the 20% starch diet the addition of metformin decreased TAG levels by 22% and 50% respectively, compared with the same diet without the drug (Fig. 6A–B). Similar results on metformin effects on TAG content were obtained by other researchers using sucrose diet (Slack et al., 2012). In mammals, the activation of AMPK by metformin stimulated oxidation of fatty acids and inhibited lipogenesis, reducing the levels of stored lipids. Lipid stores

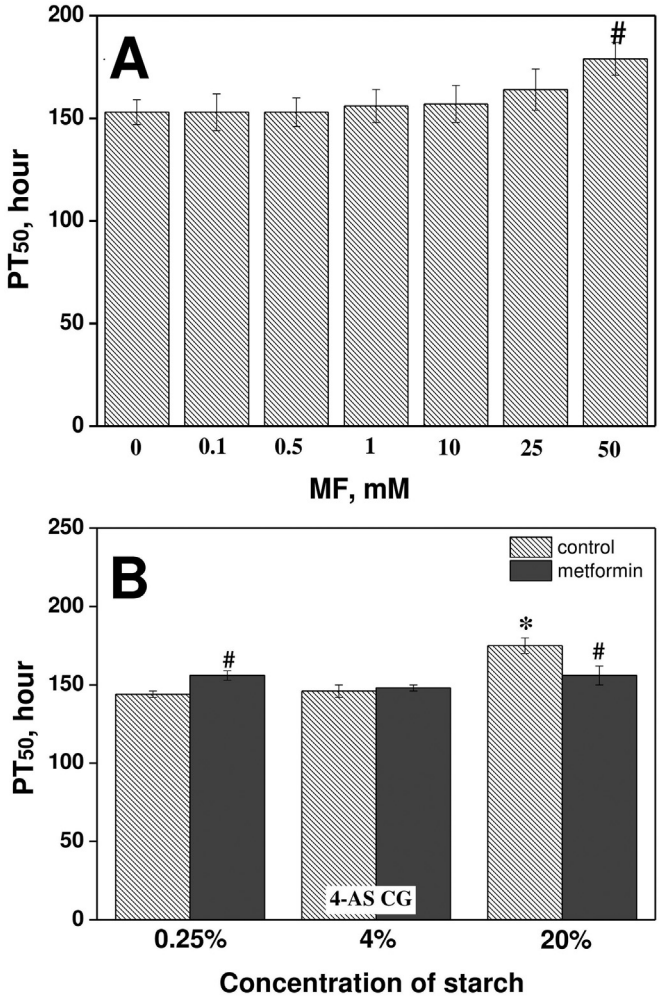

Fig. 5. Median pupariation time (PT_{50} - time at which 50% of total number larvae had pupariated) of *D. melanogaster* w^{1118} larvae raised on 4% amylose starch-containing diets containing different concentrations metformin (MF) (A) or on different starch-containing diets without or with 10 mM metformin (MF) (B). Data are presented as means ± S.E.M. In each assay cohorts with 600 individuals were used (n = 4). Significantly different from: *4% amylose starch comparison group (4-AS CG), or #control group (without MF), $P < 0.05$.

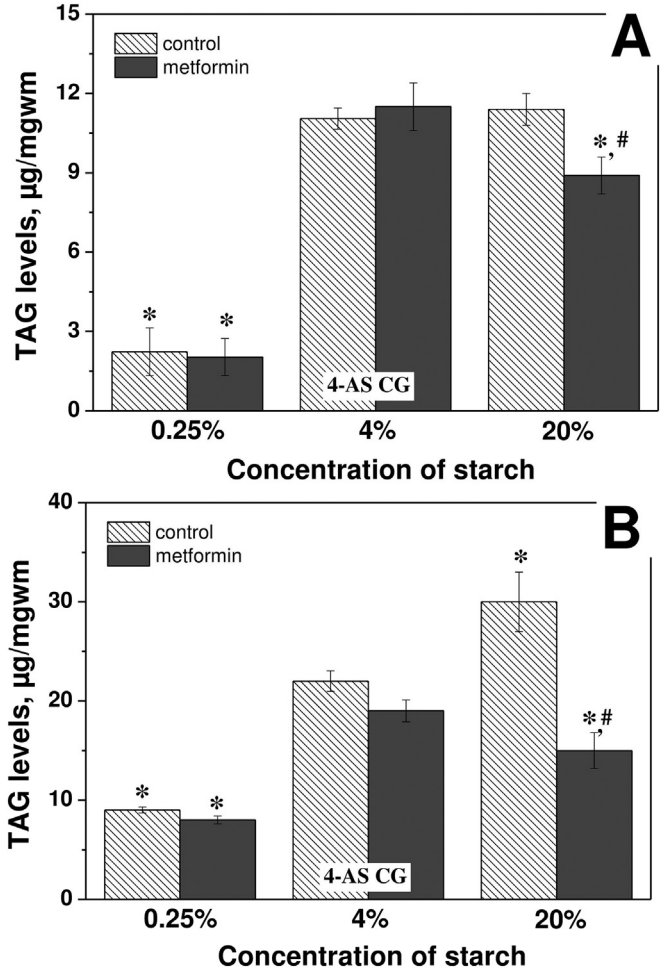

Fig. 6. The levels of triacylglycerides (TAG) in the bodies of eight day-old-male (A) and female (B) *D. melanogaster* w^{1118} flies raised on different amylose starch diets without and with 10 mM metformin (larvae were grown on 0.25–20% amylose starch diets supplemented or not with metformin and newborn flies were transferred to fresh experimental media with the same composition as they had as larvae). Data are presented as means ± S.E.M. (n = 6). Significantly different from: *4% amylose starch comparison group (4-AS CG), or #control group (without MF), $P < 0.05$.

were also reduced in nematodes (*Caenorhabditis elegans*) grown in the presence of metformin (Onken and Driscoll, 2010). Thus, metformin appears to have evolutionary conserved effects on lipid metabolism.

It was previously shown that metformin increased mean lifespan in *C. elegans* in a dose-dependent manner within the concentration range of 1 to 50 mM in the culture medium (Onken and Driscoll, 2010). In R6/2 transgenic short-lived mice, metformin increased the lifespan of males, but not of females, at a concentration of 2 mg/mL in drinking water, but not at a concentration of 5 mg/mL (Ma et al., 2007). In *Drosophila*, metformin treatment of flies on a sucrose diet at concentrations 1–25 mM had no effect on lifespan but decreased it at concentrations 50–100 mM (Slack et al., 2012). In the present study (Fig. 7), long-term consumption of 20% starch reduced the survival of both male and female flies by 50%, compared to those consuming 4% starch (median survival times are presented in Fig. 7 legend). In addition, it was previously shown that the presence of starch in the food reduced the survival of male and female flies compared to flies fed on glucose medium. Median survival time on 4% glucose and 4% starch, was 60 and 40 days for males, and 66 and 56 days for females, respectively (Abrat, 2015). Hence, it seems that amylose starch induced metabolic perturbations in fly bodies which shortened lifespan. It might be possible that: (1) amylose starch induced metabolic perturbations in fly bodies which shortened lifespan; (2) a starch diet would alter the gut microbiota, perhaps in a negative way that ultimately impacts the larvae/flies, since a high amylose starch diet is not a natural one for *Drosophila*. But these points require further investigation. Unlike the results for metabolic parameters (Figs. 4, 6), metformin at concentration of 10 mM not only did not prevent the negative consequences of a starch diet, but also decreased lifespan of both fly sexes (Fig. 7). Similar results were obtained for starch diets with the addition of metformin at lower concentrations (0.1–1 mM) (data not shown). Recently Prior and Cabreiro showed that necessary to consider the effect of drugs not only on the individual, but also on their microbiota (Pryor and Cabreiro, 2015). It has been shown, that in *C. elegans*, metformin slows down the aging process in a dose-dependent manner only if bacteria were present (Pryor and Cabreiro, 2015). Slack et al. (2012) showed that 100 mM metformin lead to changes in intestinal homoeostasis causing fluid imbalances resulting in more concentrated fecal deposits in a possible attempt to preserve water. Our results may indicate that starch and metformin influenced intestinal microbiota, but this assumption remains to be tested. The main and uniform conclusion presented herein is that metformin acts 'selectively' and its effects on fly lifespan vary depending on species and, probably, other factors, including the composition of the food medium and sensitivity of gut microbiota.

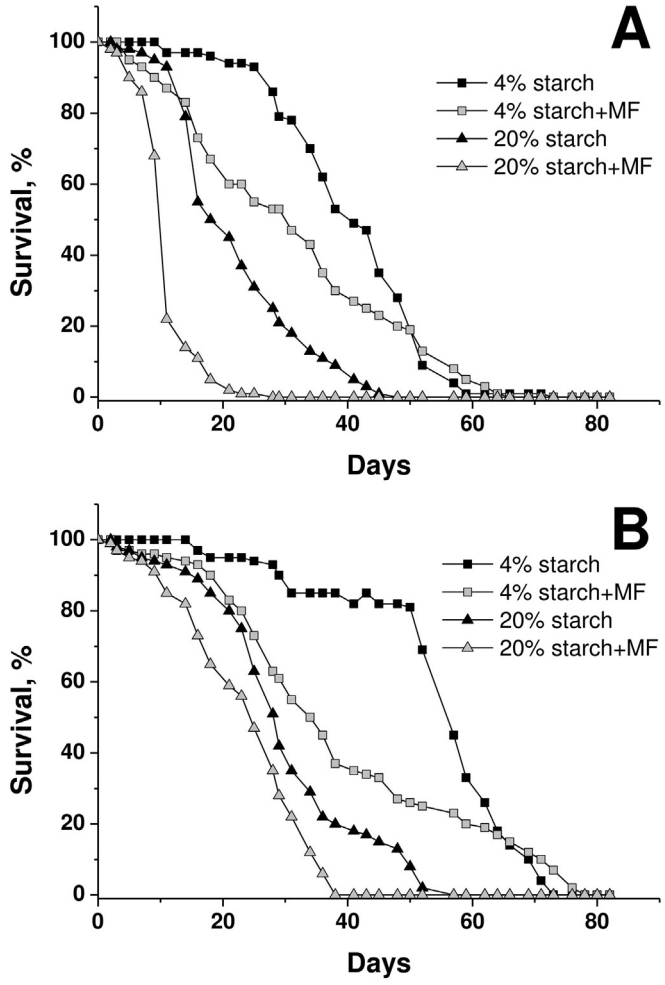

Fig. 7. Survival curves of *D. melanogaster* w^{1118} males (A) and females (B) maintained on amylose starch diets with or without 10 mM metformin (MF). For five day-old flies amylose and metformin were started. Each curve represents survivorship of about 300 flies (results are shown for one experiment and are representative of three independent experiments). Median survival time was calculated from the average survival in each of three cohorts. For males, median survival times on 4% starch, 4% starch + MF; 20% starch and 20% starch + MF were 40 ± 2 (n = 294), 30 ± 1 (n = 300), 18 ± 1 (n = 258), 10 ± 1 (n = 276) days, respectively. For females, median survival times on the same diets were 56 ± 3 (n = 282), 34 ± 2 (n = 296), 28 ± 1 (n = 289), 24 ± 1 (n = 290) days, respectively.

4. Conclusions

Our data clearly demonstrate that diets with high concentrations of amylose starch delay pupariation, shorten lifespan and cause TAG accumulation in fruit flies, *D. melanogaster*. Starch levels in larval food but not in adult food modulated triacylglyceride levels in adult flies. Food supplementation with 10 mM metformin partly attenuated the negative effects of starch on fly development and lipid content. However, prolonged metformin treatment shortened the lifespan of the insects.

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The authors declare that there are no conflicts of interest.

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