Comparative Biochemistry and Physiology, Part A 160 (2011) 27–34

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Drosophila melanogaster larvae fed by glucose and fructose demonstrate difference in oxidative stress markers and antioxidant enzymes of adult flies

Oleh V. Lushchak, Bohdana M. Rovenko, Dmytro V. Gospodaryov, Volodymyr I. Lushchak *

Department of Biochemistry and Biotechnology, Precarpathian National University named after Vassyl Stefanyk, 57 Shevchenko Str., Ivano-Frankivsk, 76025, Ukraine

ARTICLE INFO

Article history:
Received 27 January 2011
Received in revised form 27 April 2011
Accepted 27 April 2011
Available online 15 May 2011

Keywords:
Drosophila melanogaster
Fructose
Glucose
Oxidative stress markers
Antioxidant enzymes

ABSTRACT

Activities of antioxidant and associated enzymes, and oxidative stress markers were assessed in newly enclosed adult fruit flies *Drosophila melanogaster* developed on diets with 4 and 10% glucose or fructose. In fly males, 10% fructose promoted higher content of protein carbonyls and catalase activity, but lower superoxide dismutase (SOD) activity than 4%, while in females—lower levels of high molecular mass thiols (H-SH). Females at all diets had virtually the same level of lipid peroxides, low-molecular-mass thiols, catalase, and superoxide dismutase activities. Fed with 4% fructose and glucose males demonstrated 24 and 26% lower H-SH level than females, respectively. On diets with 4% glucose, 10% glucose and fructose females had 32, 26 and 27% lower catalase activity than respective males, and 1.3–1.5-fold lower glucose-6-phosphate dehydrogenase activity on glucose-containing diets. Strong positive correlations between H-SH level and G6PD activity, as well as between catalase and G6PDH activity were found. These results suggest that type and concentration of dietary carbohydrate affect antioxidant defense in fruit flies. It also substantially depends on fly sex, comprising presumably levels of protein carbonyls and lipid peroxides, as well as catalase and SOD activities in males and G6PDH activity in females.

1. Introduction

Fruit fly, *Drosophila melanogaster*, is considered to be a suitable and appropriate model organism for investigation of many aspects of animal nutrition (Bharucha, 2009). Flies and humans have similar insulin signaling machineries (Bharucha, 2009; Grewal, 2009; Teleman, 2010), and homologies between important transcriptional regulators of carbohydrate, protein and lipid metabolism, such as FoxO (Teleman, 2010), TOR (Grewal, 2009), SREBP (Theopold et al., 1996), ChREBP (Li et al., 2006), PGC-1α (Tiefenböck et al., 2010), etc. Up to now, many studies, carried out on *D. melanogaster*, have been concerned to effects of caloric or dietary restriction on lifespan, fecundity, metabolic rate and other physiological and biochemical parameters (Bross et al., 2005; Piper et al., 2005; Lee et al. 2008). Last years, *Drosophila* was examined as a model for obesity and diabetes (Skorupa et al., 2008; Al-Anzi et al., 2009; Bharucha, 2009). Carbohydrate metabolism seems received the most attention, because carbohydrates are the main component of a fruit fly natural food, and the issue on the balance between protein and carbohydrate content can be investigated in this subject also. Some recent studies have provided a comprehensive analysis for influence of under- and overnutrition on fly physiological parameters, including protein and triacylglyceride concentrations, mean and maximum lifespans (Lee et al., 2008; Skorupa et al., 2008; Simpson and Raubenheimer, 2009). Despite that, many physiological, biochemical and molecular aspects of carbohydrate overfeeding remain obscure.

Many adverse effects, connected with carbohydrate metabolism disorders, are referred to carbohydrate toxicity. Particularly, both glucose and fructose can glycate proteins. Occurring at high concentrations in tissues, these monosaccharides can be converted into toxic substances such as glyoxal or methylglyoxal (Baynes and Thorpe, 1999; Brownlee, 2005; Forbes et al., 2008; Negre-Salvayre et al., 2009). Fructose overconsumption can lead to insulin resistance, metabolic syndrome (Basciano et al., 2005; Miller and Adeli, 2008) and obesity (Tappy and Lê, 2010). Oxidative stress is thought to be one of the main mechanisms for the pathogenesis of diabetic complications (Baynes and Thorpe, 1999; Brownlee, 2005; Forbes et al., 2008; Negre-Salvayre et al., 2009). There is no clear evidence on carbohydrate capability to generate reactive oxygen species directly, but the products of carbohydrate interaction with proteins and amino acids may be generators of ROS (Semchyshyn et al., 2011). In particular, products of amino acid glycation, such as N^{ε}-carboxymethyllysine, pyralline, pentosidine, glyoxal lysine adduct, can interact with specific receptors, which activate NADPH oxidase, promoting production of superoxide anion (Baynes and Thorpe, 1999; Forbes et al., 2008; Coughlan et al. 2009; Negre-Salvayre et al., 2009). The above described processes are not attributed to normal

Abbreviations: DTNB, 5,5′-dithio-bis (2-nitro) benzoic acid; EDTA, ethylene diamine tetraacetate; G6PD, glucose-6-phosphate dehydrogenase; GSSG/GSH, oxidized/reduced glutathione; H-SH, high molecular mass thiols; LOOH, lipid peroxides; L-SH, low molecular mass thiols; $NADP^+$/NADPH, oxidized/reduced nicotine amide adenine dinucleotide phosphate; PC, protein carbonyls; PMSF, phenylmethylsulfonyl fluoride; SOD, superoxide dismutase; TCA, trichloracetic acid.

* Corresponding author. Fax: +380 3422 31574.
E-mail address: lushchak@pu.if.ua (V.I. Lushchak).

1095-6433/$ – see front matter © 2011 Published by Elsevier Inc.
doi:10.1016/j.cbpa.2011.04.019

physiological state, but mainly occur at high glucose or fructose concentrations, lack of insulin, chronic overconsumption of these carbohydrates, or postprandially. The effects of high carbohydrate diet in healthy animals are poorly understood. It is not clear if carbohydrate overfeeding would lead to a shift in redox balance and oxidative stress. In this context, the effects of glucose and fructose overconsumption on antioxidant system and oxidative damage have not been examined. Moreover, "there have been few studies directly comparing the effects of fructose to other caloric sweeteners, such as glucose, high fructose corn syrup, and sucrose" (Murphy, 2009).

In the present work, we have investigated the effects of fructose and glucose on antioxidant defense system of a model organism *D. melanogaster*. In particular, the activities of antioxidant (superoxide dismutase and catalase) and related (glucose-6-phosphate dehydrogenase) enzymes as well as levels of oxidative stress markers, namely protein carbonyl, lipid peroxide, low- and high molecular mass thiol compounds were evaluated in two days old fruit flies, grown on media with 4 or 10% either fructose or glucose. It has been supposed that chronic consumption of relatively high amounts of carbohydrates may affect antioxidant defense status of organism, and the change of this status can be related with carbohydrate concentration. Usually, *Drosophila* flies are fed on substrates containing 4–20% carbohydrates (Sang, 1956; Keller, 2007). Hence, the diet with 10% carbohydrates, used in the present study, would not be a substantial overconsumption of carbohydrates. However, the difference between concentrations let us to detect a direction of changes in the parameters of interest.

2. Materials and methods

2.1. Reagents

Phenylmethylsulfonyl fluoride (PMSF), oxidized glutathione (GSSG), $NADP^+$, NADPH, glucose-6-phosphate, ethylenediaminetetraacetic acid (EDTA), xylenol orange, cumene hydroperoxide, ferrous sulfate, 2,4-dinitrophenylhydrazine (DNPH), N,N,N′,N′-tetramethylethylenediamine (TEMED), Tris–HCl, 5,5′-dithio-bis (2-nitro) benzoic acid (DTNB), were purchased from Sigma-Aldrich Chemie GmbH (Germany), guanidine-HCl was from Fluka. All other reagents were of analytical grade.

2.2. Flies and experimental design

The laboratory wild-type stock IF was collected in Ivano-Frankivsk in August, 2007. Flies were reared on yeast-molasses meal contained 6% (w/v) yeast, 4% (v/v) molasses, 1.3% (w/v) agar-agar and 0.4% (v/v) propionic acid. For egg collection, parental flies were transferred for 18 h onto appropriate meal contained 4% yeast, 4 or 10% (w/v) fructose or glucose, 1.3% (w/v) agar-agar and 0.4% (v/v) propionic acid. Received eggs were counted, and then 260–280 eggs were plated onto respect meal (25 ml of meal in 250 ml flasks). Newly-eclosed flies were transferred into the flasks with appropriate meal and held for two days. Two days old flies were used for biochemical analyses. Ten flies of each gender were routinely used for determination of lipid peroxide content, while groups of 50 individuals were used for preparation of homogenate pool in which protein carbonyl, low- and high molecular mass thiol-containing compound content, and enzyme activity were measured. Depending on the parameter, each assay was performed in 3–10 independent replicates (details are presented in legends to figures).

2.3. Food intake

Groups of 10 3rd instar larvae were reared on the abovementioned media and placed for 20 min on the same food with 0.5% dye FD&C Blue No. 1 (Brilliant Blue FCF) poured on Petri dishes. After feeding, each group of larvae was immediately frozen in liquid nitrogen. Further larvae were then homogenized in 100 μl of 50 mM potassium phosphate (KPi) buffer, pH 7.5, centrifuged at 13,500 *g* for 15 min, and supernatant was transferred to a new tube containing 100 μl of the buffer for dilution. Diluted samples were measured in a 96-well microplate reader at 629 nm. The dye diluted in 50 mM KPi buffer was used to build a calibration curve. Optical density of the homogenates from larvae consumed corresponding diets without the dye was used as a blank.

2.4. Oxidative stress markers

Flies were separated into sexes, weighted and homogenized (1:10 w/v) with Potter-Elvjeham glass homogenizer in 50 mM KPi buffer, pH 7.5, containing 0.5 mM EDTA and 1 mM PMSF. Samples were centrifuged for 15 min at 13,000 *g* (Eppendorf 5415R) and resulted supernatants were used for analyses.

All oxidative stress markers were measured as described previously (Lushchak et al., 2009a). Content of carbonyl groups in proteins (PC) was measured detecting the amount of 2,4-dinitrophenylhydrazone formed upon the reaction with DNPH. Protein carbonyl content was calculated from the absorbance maximum of 2,4-dinitrophenylhydrazone measured at 370 nm using an extinction coefficient of 22 mM^{-1} cm^{-1}. The results were expressed in nanomoles per milligram of protein.

Lipid peroxide (LOOH) content was assayed with xylenol orange (Hermes-Lima et al., 1995). For that, flies were homogenized with 10 volumes of 96% (vol.) cold (~5 °C) ethanol, centrifuged for 5 min at 13,000 *g*, and supernatants were used for assay. The content of LOOH is expressed as nanomoles of cumene hydroperoxide equivalents per gram of fly weight. Free thiols were measured spectrophotometrically with DTNB at 412 nm. Total thiol content (the sum of low- and high-molecular-mass thiol-containing compounds) was measured in the resulting supernatants. For measurement of low-molecular-mass thiol-containing compounds (L-SH) content, supernatants were treated with 10% TCA (final concentration), centrifuged for 5 min at 13,000 *g* and the final supernatants were used for the assay. The thiol concentrations were expressed as micromoles of SH-groups per gram of fly wet weight. For weighting flies balances WTW2 ("Techniprot", Poland) with accuracy 0.2 mg were used.

2.5. Enzyme activities

Enzyme activities were measured as described earlier (Lushchak et al., 2005b). Briefly, SOD activity was assayed at 406 nm as inhibition of quercetin oxidation by superoxide anion. One unit of SOD activity was defined as the amount of soluble protein of supernatant that inhibited the maximum rate of quercetin oxidation by 50%. Glucose-6-phosphate dehydrogenase (G6PDH) activity was measured spectrophotometrically at 340 nm by the rate of NADPH production. Extinction coefficient 6.22 mM^{-1} cm^{-1} for NADPH was used. Catalase activity was registered as a rate of hydrogen peroxide decomposition at 240 nm, using 39.4 M^{-1} cm^{-1} as extinction coefficient for hydrogen peroxide (Nelson and Kiesow, 1972). All reactions were started by addition of fly homogenates. One unit of G6PDH and catalase activities was defined as the amount of supernatant protein that produces or utilizes 1 μmol of corresponding product or substrate per minute, respectively. All activities were measured at 25 °C and expressed per milligram of soluble protein in supernatant.

2.6. Protein concentration estimation and statistical analysis

Protein concentration was measured by the Bradford method with Coomassie Brilliant Blue G-250 (Bradford, 1976) using bovine serum albumin as a standard. Data are presented as means ± S.E.M and compared by unpaired 2-tailed Student's test. Correlation analysis