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Restriction of glucose and fructose causes mild oxidative stress independently of mitochondrial activity and reactive oxygen species in *Drosophila melanogaster*

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ABSTRACT

Our recent study showed different effects of glucose and fructose overconsumption on the development of obese phenotypes in *Drosophila*. Glucose induced glucose toxicity due to the increase in circulating glucose, whereas fructose was more prone to induce obesity promoting accumulation of reserve lipids and carbohydrates (Rovenko et al., Comp. Biochem. Physiol. A Mol. Integr. Physiol. 2015, 180, 75–85). Searching for mechanisms responsible for these phenotypes in this study, we analyzed mitochondrial activity, mitochondrial density, mtROS production, oxidative stress markers and antioxidant defense in fruit flies fed 0.25%, 4% and 10% glucose or fructose. It is shown that there is a complex interaction between dietary monosaccharide concentrations, mitochondrial activity and oxidative modifications to proteins and lipids. Glucose at high concentration (10%) reduced mitochondrial protein density and consequently respiration in flies, while fructose did not affect these parameters. The production of ROS by mitochondria did not reflect activities of mitochondrial complexes. Moreover, there was no clear connection between mtROS production and antioxidant defense or between antioxidant defense and developmental survival, shown in our previous study (Rovenko et al., Comp. Biochem. Physiol. A Mol. Integr. Physiol. 2015, 180, 75–85). Instead, mtROS and antioxidant machinery cooperated to maintain a redox state that determined survival rates, and paradoxically, pro-oxidant conditions facilitated larva survival independently of the type of carbohydrate. It seems that in this complex system glucose controls the amount of oxidative modification regulating mitochondrial activity, while fructose regulates steady-state mRNA levels of antioxidant enzymes.

1. Introduction

Mitochondria generate most of ATP that cells use to fuel their metabolism and other activities (Haslam and Krebs, 1968; Kang and Pervaiz, 2012). Apart from providing cells with energy, mitochondria are involved in various important cellular processes such as beta-oxidation of fatty acids and biosynthesis of pyrimidines, amino acids, nucleotides, phospholipids, hemes, and iron–sulphur clusters. Concomitantly, mitochondria are also the main generators of reactive oxygen species (ROS) (Weinberg et al., 2010; Jimenez-Del-Rio and Velez-Pardo, 2012; Kang and Pervaiz, 2012). It was known that increased steady-state ROS concentration may lead to modification of proteins, lipids, and nucleic acids which along with certain physiological consequences reflect development of oxidative stress (Lushchak, 2011, 2014; Scialo et al., 2013). During the last decade, understanding of relationship between intermediary and ROS metabolisms is notably increased. Recent studies showed that ROS are not only damaging factors, but also play regulatory roles in cell fate determination, hypoxia response, apoptosis, and necrosis (Sena and Chandel, 2012; Filomeni et al., 2015). In addition to the "classical ideas" about oxidative damages that postulated an irreversible pattern of biomolecule damages, nowadays substantial piece of evidence has been accumulated clearly indicating that oxidatively modified molecules might be repaired (Clancy and Birdsall, 2013), or removed by the proteasome (Pickering and Davies, 2012) and autophagy (Filomeni et al., in press).

Abbreviations: G6PDH, glucose-6-phosphate dehydrogenase; GR activity of TrxR, glutathione reductase activity of thioredoxin reductase; GST, glutathione-S-transferase; IDH, isocitrate dehydrogenase; MD, mitochondrial protein density; mtROS, mitochondrial ROS; PC, protein carbonyls; PT, protein thiols; ROS, reactive oxygen species; SOD, superoxide dismutase.

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Despite ROS being constantly generated in living organisms, under "normal" conditions organisms are able to tolerate them. In fact, oxidative stress only happens, when the balance between ROS production and elimination is disturbed resulting in increased ROS levels along with certain physiological consequences (Lushchak, 2011; Scialo et al., 2013). Oxidative stress accompanies many disorders including diabetes, atherosclerosis, cancer, and degenerations, which also depend on type of diet, for example, availability of carbohydrates or amino acids (Sanz et al., 2006; Weinberg et al., 2010; Henriksen et al., 2011; Hulsmans et al., 2012; Jimenez-Del-Rio and Velez-Pardo, 2012).

Studies in mammalian models indicated that reducing monosaccharides, glucose and fructose, differently affect mitochondrial function and antioxidant defense (Rizkalla, 2010; Kunde et al., 2011; Shah et al., 2013). However, it is unknown if these changes in antioxidant defense are caused by changes in mitochondrial activity and/or ROS levels. Nowadays, Western countries suffer an epidemic of obesity caused to a large extent by overconsumption of carbohydrates. It is believed that part of the problems is related with the increased intake of glucose and fructose (Basciano et al., 2005; Moeller et al., 2009; Tappy and Lê, 2010; Stanhope et al., 2013). Both, glucose and fructose may stimulate ROS generation via non-enzymatic reactions also known as glycation reactions causing oxidative stress (Kanska and Boratynski, 2002; Schalkwijk et al., 2004; Semchyshyn, 2013, 2014; Semchyshyn et al., 2014). However, it remains unclear if there is any difference in their capability to initiate glycation, since some reports call glucose (Kanska and Boratynski, 2002), whereas others call fructose as a more powerful glycation agent (Bunn and Higgins, 1981; Schalkwijk et al., 2004; Semchyshyn et al., 2011; Semchyshyn, 2013). It is also controversial if excessive consumption of monosaccharides causes oxidative damage via glycation (Schalkwijk et al., 2004; Shangari and O'Brien, 2004; Semchyshyn et al., 2011; Semchyshyn, 2013) or through changes in mitochondrial metabolism (Rizkalla, 2010; Shah et al., 2013; Mortensen et al., 2014). The time scales for these processes seem to be different: if glycation is considered to be rather slow process (Semchyshyn, 2013, 2014), mitochondria may quickly respond to alterations in dietary conditions (Barja, 2007; Scialo et al., 2013). Previous studies from our laboratory (Lushchak et al., 2011; Semchyshyn et al., 2011, 2014; Semchyshyn and Lozinska, 2012; Rovenko et al., 2013; Semchyshyn, 2014) indicated the complex relationship between fructose consumption and ROS-related processes. For example, fructose at high concentrations promoted protein oxidation, while at moderate concentrations it protected *Saccharomyces cerevisiae* cells from oxidative damage inducing a hormetic response (Semchyshyn et al., 2011, 2014; Semchyshyn and Lozinska, 2012). Similar data were obtained in *Drosophila melanogaster*, where glucose and fructose demonstrated different effects depending on the genetic background and the stage of the life cycle studied (Lushchak et al., 2011, 2014; Rovenko et al., 2013). Carbohydrates are metabolized through similar pathways in flies and mammals (Kunieda et al., 2006; Zera, 2011). In flies, neurosecretory cells from the brain and *corpora cardiaca* as well as fat body are instrumental to maintain energy homeostasis, playing the same role that pancreas, liver and adipose tissue in vertebrates (Teleman et al., 2012; Owusu-Ansah and Perrimon, 2014). Although during the past decade substantial progress has been made in understanding carbohydrate metabolism regulation, the energy control upon feeding with different carbohydrates, especially fructose, remains unclear. Recent findings showed that brain possesses a specific receptor for fructose (Gr43a), which is able to sense fructose from circulating sugars regulating feeding behavior (Miyamoto et al., 2012). Several independent studies showed strong preferences of *Drosophila* to fructose-enriched food (Masek and Scott, 2010; Lushchak et al., 2011; Mishra et al., 2013; Rovenko et al., 2015). Our latest study (Rovenko et al., 2015) showed that metabolic response to fructose overfeeding in flies mimics features of the response in mammals (reviewed in details in Basciano et al., 2005; Tappy and Lê, 2010). Thus, fructose rather than glucose promoted a diet-induced obese phenotype partly by modulating the insulin/insulin-like growth factor signaling. Glucose overfeeding, in turn, induced glucose toxicity and decreased developmental survival due to the increase in circulating glucose (Rovenko et al., 2015). In this study, we expanded our understanding of the role of glucose and fructose in the regulation of mitochondrial function in *Drosophila*, and described possible implication of mitochondrial function to observed phenotypes. In particular, we were interested to know: (1) how mitochondrial activity is modified in response to variation in dietary monosaccharide composition and concentration; (2) how changes in mitochondrial function affect mitochondrial ROS production; (3) how changes in mitochondrial ROS metabolism are related to ROS-promoted oxidative modifications of lipids and proteins, and (4) how free radical metabolism interfere with glucose- and fructose-derived metabolic phenotypes. For this, we fed *Drosophila* flies during development with glucose and fructose, in different concentrations, and analyzed mitochondrial activity, mitochondrial density, and ROS production, along with oxidative stress indices and antioxidant response in young adults.

2. Materials and methods

2.1. Reagents

All reagents were purchased from Sigma-Aldrich Corporation (USA) unless otherwise stated. RNA stabilization solution was obtained from Ambion (USA). Oligonucleotides for Q-RT-PCR (qPCR) assay were purchased from TAG Copenhagen A.S. (Denmark). SensiFAST SYBR Hi-ROX Kit was obtained from Bioline Reagents Ltd. (United Kingdom). The other reagents for qPCR assay were from Thermo Fisher Scientific (USA). Manufacturer yeasts (type "Extra", TM "The Lviv yeast") were bought from "The Enzyme Company" (Ukraine).

2.2. Flies and experimental design

Wild type *Canton S* flies were used in all experiments. The flies were from Bloomington Drosophila Stock Center at Indiana University (USA). Flies were reared on medium containing 6% (w/v) yeasts, 4% (v/v) molasses, 1.25% (w/v) agar and 0.4% (v/v) propionic acid as a mold growth inhibitor. For egg collection, about 300–400 of 3–7 day-old parental flies were transferred into demographic cages with the open side attached to a Petri dish containing egg collection medium (apple juice with 2% agar and yeast paste). After 18 h eggs were washed out from the egg collection plate with distilled water and counted. About 260–280 eggs were placed into 250 ml glass flasks containing 25 ml of experimental food with 4% yeast, 0.25, 4, or 10% glucose or fructose, 1.25% (w/v) agar, and 0.4% (v/v) propionic acid. The caloric values of diets were calculated by counting calories derived from sucrose and yeast. 4 kcal/g was used as sucrose caloric value (Donato, 1987). The caloric value of yeast was taken as 1.17 kcal/g according to manufacturer's annotation. Total caloric content of the diets is presented in Suppl. Table 1. In natural environment the concentrations of glucose and fructose in *Drosophila* food varies from 2 to 30% with an average around 4–6% in most fruits (Widdowson and McCance, 1935; Li et al., 2002). According to this information and our previous data concerning developmental survival and food consumption with glucose and fructose (Rovenko et al., 2015), the diet with 0.25% carbohydrate was considered as a carbohydrate restricted diet, while diets with 4 and 10% of monosaccharides were suggested to yield relatively moderate and high concentrations of carbohydrates in the *Drosophila* food, respectively.

Newly eclosed flies were transferred onto fresh food of the same composition, where they have been growing during larval stage and held for two days. Two-day old flies were separated by sex and used for biochemical analyses.