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Attenuation of high sucrose diet–induced insulin resistance in ABC transporter deficient *white* mutant of Drosophila melanogaster

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Abstract

Exposure to high sugar diet (HSD) is an experimental model of insulin resistance (IR) and type 2 diabetes (T2D) in mammals and insects. In Drosophila, HSD-induced IR delays emergence of pupae from larvae and eclosion of imago from pupae. Understanding of mechanisms of IR/T2D is essential for refining T2D prevention and treatment strategies. Dysregulation of tryptophan (Trp)-kynurenine (Kyn) pathway was suggested as one of the mechanisms of IR/T2D development. Rate-limiting enzyme of Trp-Kyn pathway in Drosophila is Trp 2,3-dioxygenase (TDO), an evolutionary conserved ortholog of human TDO. We previously reported attenuation of HSD-induced IR in *vermilion* mutants with inactive TDO. Conversion of Trp to Kyn is regulated not only by TDO activity but by intracellular Trp transport via ATP-binding cassette (ABC) transporter encoded by white gene in Drosophila. In order to evaluate the possible impact of deficient intracellular Trp transport on the inducement of IR by HSD, we compared the effect of HSD on pre-imago development in wild type flies, Canton-Special (C-S), and C-S flies containing *white* gene, *white* (C-S). Presence of *white* gene attenuated (by 50%) HSD-induced delay of pupae emergence from larvae and female and male imago eclosion from pupae. Present study together with our earlier report reveals that both decreased TDO activity (due to *vermilion* gene mutation) or deficient Trp transport into cell without affecting TDO levels (due to *white* gene mutation) attenuate HSD-induced development of IR in Drosophila model of T2D. Our data provide further support for hypothesis that dysregulation of Trp-Kyn pathway is one of the pathophysiological mechanisms and potential target for early diagnosis, prevention and treatment of IR/T2D.

Keywords

white mutants; ABC-transporter; drosophila; high sugar diet; insulin resistance; type 2 diabetes; kynurenine; tryptophan; obesity

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Introduction

Diabetes mellitus is a public health problem, which affects a millions worldwide. Most diabetes cases are classified as type 2 diabetes mellitus (T2D), which is highly associated with obesity [1]. Impaired glucose tolerance (pre-diabetes) is a high-risk factor for T2D: up to 70% of individuals with prediabetes eventually develop T2D [2]. Insulin resistance (IR) is associated with prediabetes before T2D could be diagnosed. Therefore, understanding of mechanisms of IR is essential for developing T2D prevention and treatment strategies. Dysregulation of kynurenine (Kyn) pathway of tryptophan (Trp) metabolism was suggested as one of the mechanisms of development of IR and T2D [3–8]. We found IR correlation with activity of Trp-Kyn pathway in Hepatitis C virus (HCV) patients [9], and elevation of plasma concentrations of Kyn downstream metabolites in T2D patients [10]. Experimental model of T2D was established in Drosophila *melanogaster* [11]. There are four distinct stages in the life of *flies*: egg, larva, pupa, and imago (adult). High sugar diet (HSD) induces IR in larva and T2D in imago [12]. IR delays emergence of pupae from larva and imago eclosion from pupae [13]. In Drosophila, tryptophan 2,3-dioxygenase (TDO), enzyme, catalyzing formation of Kyn (via N-formyl-kynurenine) from Trp, is encoded by *vermilion* gene [14]. We found attenuation of HSD-induced development of IR in *vermilion* mutants with inactive TDO [15]. TDO is substrate-activated intracellular enzyme. Therefore, conversion of Trp to Kyn is regulated not only by TDO activity but by Trp transport into cells as well [16]. Import of Trp into cell is mediated by ATP-binding cassette (ABC) transporters encoded by *white* gene in Drosophila. *White* mutations do not alter levels of TDO, but interfere with the ability of cells to take up Trp [17]. Therefore, mutations of both TDO (*vermilion*) and ABC transporter (*white*) genes have a similar effect, i.e., decreased formation of Kyn from Trp. In order to evaluate the possible impact of impairment of intracellular Trp transport on the inducement of IR by HSD, we compared the effect of HSD on pre-imago development in wild type flies, Canton-Special (C-S), and C-S flies containing *white* gene, *white* (C-S).

Materials and methods

C-S wild type Drosophila melanogaster flies and *white* (C-S) mutants from the collection of V.N. Karazin Kharkiv National University were maintained at 23°C in a 12:12 light: dark period on a standard nutrition medium consisting of sugar, yeast, agar and semolina. Experimental eggs were obtained from parents with synchronized egg laying. Sucrose (0.67M) was added to nutrition medium before eggs laying. Emerging time was taken as the period from the time of synchronized egg laying to the time of larvae emergence into pupae as described elsewhere [15]. Appearance of female and male imago from pupae was recorded as a time of eclosion. The study was carried out in November and December 2015.

Statistical analysis

Data were expressed as mean±standard deviation (hours of pupae emergence and imago eclosion). Differences between experimental groups were evaluated by Mann Whitney, two-tailed test.

Results

Pupae emergence from larva

Emergence time of C-S wild type flies maintained on standard nutrition medium was 7% shorter than emergence time of *white* (C-S) mutants ($p<0.0001$) (Table 1). HSD delayed pupae emergence from larva of wild type flies, C-S, in comparison with flies maintained on standard nutrition medium by almost 3 days (40%). In *white* (C-S) mutants HSD delayed pupae emergence from larvae by 1.6 days (20%) in comparison with flies maintained on standard nutrition medium. Therefore, presence of *white* gene attenuated the delay (induced by HSD) of pupae emergence from larvae by 50% (Table 1).

Imago eclosion from pupae

There was no significant gender effect on imago eclosion time (Table 2). Eclosion times of both female and male C-S wild type flies maintained on standard nutrition medium were about 8% shorter than eclosion times of *white* (C-S) mutants ($p<0.0001$). HSD delayed imago eclosion in C-S flies in comparison with flies maintained on standard nutrition medium by 2.75 days (20.5%) in females and by 2.60 days (19.8%) in males. HSD delayed imago eclosion in *white* (C-S) mutants by 1.3 days (9%) days in females and by 1 day (7%) in males flies. Therefore, presence of *white* gene attenuated the delay (induced by HSD) of imago eclosion by 50% in comparison with wild type flies (Table 2).

Discussion

Present data indicate that HSD delays pupae emergence from larva and imago eclosion in wild type C-S flies in according with literature data [11–13]. The main finding of the present study is that HSD-induced delay of pre-imaginal development (from egg through larva and pupa to imago) was two times shorter in *white* (C-S) mutants than in wild type C-S flies. We are not aware of studies of the effect of HSD on preimaginal stages of ABC transporter deficient *white* flies. Considering that HSD-induced delay of pre-imaginal development is caused by IR [11–13], our data suggest that mutation of *white* gene attenuates HSD-induced IR.

Attenuation of HSD-induced IR in *white* (C-S) flies most likely depends on deficiency of ABC transporter that mediates Trp import into cell, and, thus, decreases availability of Trp, a substrate for intracellularly located TDO [16]. Decreased availability of Trp as a substrate for TDO results in decreased Kyn formation from Trp, without affecting TDO activity [17]. TDO-regulated KYN formation from Trp begins at the end of the third larval instar in the cells of the anterior region of fat body (analog of liver and fat tissues in humans) [14]. Immediate metabolic response to HSD in Drosophila is an increase of glucose in hemolymph [12]. Hyperglycemia induces production of O_2^* and H_2O_2, and generation of mitochondrial reactive oxygen species in rodents [18], and activates hypothalamic-pituitary-adrenal axis and, consequently, increases secretion of cortisol, a hormonal inducer of TDO, in rat model of T2D [19] and in T2D patients [20]. Notably, high glucose selectively inhibits ABC transporter G1 subtype, involved in Trp transport, in human macrophages [21]. Therefore, mutation of *white* gene, that prevents Trp import into cell, might confer resistance

to HSD-induced IR and T2D, an aging associated disease [4]. Notably, we reported pronged life span in *white* mutants [22] while resistance to high glucose-induced oxidative stress was associated with longevity in rodents [18]. Considering that *white* mutants have deficient transport of both Trp and guanine, attenuation of HSD-induced IR might depend not only on Trp but guanine transporter deficiency as well. While such a possibility could not be ruled out based on available data, some other factors might affect functions of *white* mutant, the parent strain of Methuselah Drosophila [23,24]. Further studies, e.g., of the effect of ABC-transporter inhibitor, 5-methylTrp, [25] on HSD-induced IR might help to differentiate between impairment of guanine- and Trp-ABC transporters.

Obesity is highly associated with T2D [26]. The mechanisms of such association are not clear. Dysregulation of Trp-Kyn pathway in obesity was suggested [3,6] and supported by clinical and experimental data [27–29]. It is noteworthy that in Drosophila HSD induces not only IR/T2D but obesity as well [11]. Future studies of HSD-induced obesity in *white* mutants might provide better understanding of the role of Trp–Kyn pathway in mechanisms of obesity and T2D.

In conclusion, present study together with our earlier report revealed that both decreased TDO activity (due to *vermilion* gene mutation) or deficient Trp transport into cell without affecting TDO levels (due to *white* gene mutation) attenuate HSD-induced development of IR in Drosophila model of T2D. Our data provide further support for hypothesis that dysregulation of Trp-Kyn pathway is one of the pathophysiological mechanisms and potential target for early diagnosis, prevention and treatment of IR/T2D [4–7].

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Table 1

Effect of high sucrose diet on time of pupae emergence from larvae in *white* (C-S) and Canton-S flies.

Genotype and treatment	Standard Diet	High Sucrose Diet	Delay of emergence (%)
Canton-S	182.9 ± 18.9 (n=516)	253.8 ± 24.5$^{\#}$ (n=465)	40
***white* (C-S)**	195.4 ± 22.2* (n=385)	234.2 ± 31.6$^{*\#}$ (n=261)	20

Mean ± standard deviation (hrs); n: number of pupae

Mann-Whitney two tailed test:

* p=0.0001 in comparison with Canton-S

$^{\#}$ p=0.0001 in comparison with Standard diet.

Table 2

Effect of high sucrose diet on imago eclosion *white* (C-S) and Canton-S flies.

Genotype and treatment	Standard Diet	High Sucrose Diet	Delay of eclosion (%)
Canton-S:			
Female	322.0 ± 17.1 (n=144)	388.1 ± 23.9 * (n=144)	20.5
Male	327.8 ± 16.7 (n=126)	392.7 ± 24.1 * (n=111)	19.8
***white* (C-S):**			
Female	349.3 ± 36.3 ** (n=165)	380.5 ± 32.1 * (n=108)	9
Male	357.9 ± 35.2 ** (n=163)	382.7 ± 33.6 * (n=114)	7

Mean ± standard deviation (hrs); n: number of imago.

Mann-Whitney two tailed test:

* p=0.0001 in comparison with flies on standard diet

** p=0.0001 in comparison with Canton-S