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ALPHA-KETOGLUTARATE INDUCES NUCLEAR RECEPTORS RATHER THAN NRF2 IN THE FRUIT FLY *Drosophila melanogaster*

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Alpha-ketoglutarate (AKG) is an important metabolite of the tricarboxylic acid cycle that plays a key role in the energy metabolism of an organism [1]. It was shown that AKG helps prevent aluminum toxicity and increases lifespan of the fruit fly *Drosophila melanogaster* [2, 3]. We assumed that the abovementioned effects of AKG could be mediated by activation of the nuclear factor erythroid 2-related factor 2 (Nrf2). Indeed, this factor is often associated with lifespan extension and may also provide resistance to toxins, such as metal salts [4]. Genes *Ugt37A2*, *GstD2* and *Cyp6a2* that encode uridine diphosphate glycosyltransferase family 37 member A2, glutathione *S*-transferase D2, and cytochrome P450 6a2, respectively, are important targets of Nrf2 that play a key role in xenobiotic detoxification in *D. melanogaster* [5]. Expression levels of these genes can be markers of Nrf2 activation by either substance, including AKG.

Aim. To test whether expression of Nrf2 targets in *D. melanogaster* is activated by AKG exposure.

Methods. The *Canton-S* strain of the fruit fly *Drosophila melanogaste*r was used in the study. Flies were grown on a standard medium containing 5% sucrose, 5% commercial baker's yeast, 6.1% corn grits, 1% agar-agar, 0.18% methyl 4-hydroxybenzoic acid (nipagin) as a mold growth inhibitor. Four-day-old females (150 flies per group) were placed into demographic cages with the medium containing 5% sucrose, 5% yeast, 1.2% agar, 0.18% nipagin. Experimental diet was supplemented with 10 mM disodium salt of AKG. Flies were kept on these media for 21 days. Then, the flies were anesthetized with carbon dioxide and snap-frozen in liquid nitrogen for further biochemical studies.

The levels of messenger ribonucleic acid (mRNA) levels were determined using reverse transcription polymerase chain reaction (RT-PCR) followed by detection of products in agarose gel. Messenger ribonucleic acid was purified using Monarch® Total RNA Miniprep kit (New England Biolabs (NEB), T2010). Expression of genes *Ugt37A2*, *GstD2*, *Cyp6a2*, and *Tbp* (TATA-box-binding protein), as a reference gene, was evaluated. After ethidium bromide staining, the gels were scanned, and the intensity of the bands was measured in arbitrary units using the ImageJ program. The values in the graph were expressed as the ratio of the experimental genes to the *Tbp* gene.

Results and Discussion. Continuous consumption of AKG-supplemented food resulted in 2.8-fold increase in the mRNA levels of *Cyp6a2* gene compared to the control group (Figure). At the same time, the expression of *Ugt37A2* and *GstD2* genes in the individuals of the control group and the group that consumed the diet with AKG did not differ significantly.

Activation of the *Cyp6a2* expression by AKG suggested that the latter might influence Nrf2. However, AKG-supplemented food did not affect expression of other established targets of Nrf2. This implied that AKG might affect other transcriptional regulators of xenobiotic response than Nrf2. In particular, *Cyp6a2* was found to be regulated by the nuclear receptor DHR96, a homolog of human pregnane X and constitutive androstane receptors (6). It was previously shown that DHR96 was involved in metabolism of triacylglycerols and might partially be involved in the lifespan regulation (7, 8).

Conclusions. Continuous consumption of AKG-supplemented food resultyв in the increase in mRNA levels of *Cyp6a2* gene, a target of transcriptional factors Nrf2 and DHR96, but not *Ugt37A2*

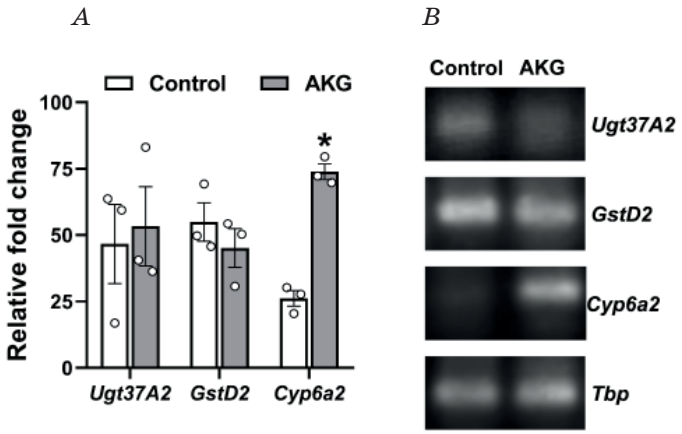

Fig. **Relative levels of mRNA of the genes studied in the body of 25-day-old fly fed on the control medium and the medium supplemented with 10 mM AKG (*A*) and representative PCR gels**
Asterisk denotes statistically significant differences (Student's *t*-test, $P < 0.05$, $n = 3$)

and *GstD2* genes. Since expression of the latter two genes was unaffected by AKG-supplemented diet, it indicated that AKG might influence other transcriptional regulators, such as nuclear receptors that had common targets with Nrf2.

Key words: *Drosophila melanogaster*, alpha-ketoglutarate, mRNA, *Cyp6a2*, Nrf2.

Authors' contribution. OID carried about fruit fly cultures, assisted in the experiments, conducted calculations, and wrote the original draft of the abstract, DVG performed PCR, reviewed and edited the abstract.

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