PHYTOCHEMICAL SCREENING IN DROSOPHILA MELANOGASTER

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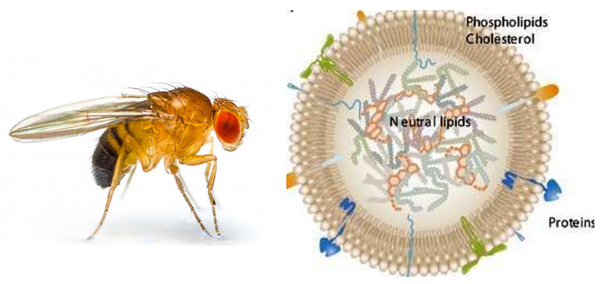
Introduction

The fruit fly *Drosophila melanogaster* has been extensively studied for over a century as a *model* organism for *genetic* investigations, and over the past four decades has become a powerful tool for analyzing the function of human disease genes. Many basic biological and physiological processes are conserved between mammals and *Drosophila*, and nearly 75% of human disease-causing genes are believed to have a functional homolog in the fly. The highly conserved disease and biological pathways between human and *Drosophila* and the rapid life cycle, low cost and easy manipulation make *Drosophila* an ideal organism for screening compounds. Traditionally, drug screening processes are based on *in vitro*, enzymatic or receptor binding assays, but lead compounds identified are often ineffective or toxic after *in vivo* test. The fruit fly represents one such valid alternative in the drug discovery process. [1]

Lipid droplets (LDs) are complex and dynamic cytosolic organelles whose function is to assemble, store, and supply neutral lipids, mainly sterol esters and triacylglycerols (TAGs). PPAR, RXR, SREBP, DGAT1, DGAT2 (named minotaur and midway in *Drosophila*) are the main receptors and enzymes involved in human LDs biogenesis and they all have a homolog in *Drosophila.* Defects in LDs biogenesis/turnover during excess or deficient fat storage lead to many different human diseases such as cancer, lipodystrophies, cardiovascular and neurodegenerative disorders [1]. The biological and physiological role of LDs in muscle and nervous system as well as their pharmacological modulation are unknown. Here we used *Drosophila* as an *in vivo* model for drug screening of LDs modulators.

Aim

The aim of this study was to verify the effects of different compounds on lipid droplets biogenesis in specific tissues such as muscles and axons *in vivo*.



Experimental design

In this work, we analyzed the *in vivo* modulation of LDs biogenesis after administration of phytochemicals in the food of the wild type *Drosophila* strain White^{1118}. We tested three main classes of compounds: modulators of PPAR, RXR, and SREBP. The number and size of lipid droplets in muscles and axons, as well as the gene expression of receptors and enzymes involved in lipid metabolism were quantified.

Results

RXR Modulators

Compounds	Class	Action	Origin
Retinoic acid	Retinoid	RARs and RXRs activator	Vitamin A derivated
Bexarotene	Rexhinoid	RXRs agonist	Vitamin A derivated
Isoquercetin	Cholic acid s	FXR agonist	Synthetic

PPARs Modulators

Compounds	Class	Action	Origin
Naringenin	Flavanone	PPARα and PPARγ agonist	Grapefruit seeds
Hesperitin	Flavanone	PPARγ agonist	Orange peel
Isoquercetin	Flavonoid	PPARγ agonist	Bark of Salix
Fenofibrate	Benzophenone derivate	PPAR α agonist	Synthetic
Genistein	Isoflavone	PPARα and PPARγ agonist	Soy
β-Estradiol	Oestrogen	Anti-hoxhidant and neuroprotctive hormone	Hormone
GW 6471	L-tyrosine analog	PPARα antagonist	Synthetic

SREBP Modulators

Compounds	Class	Action	Origin
Xanthohumol	Flavonoid	SREBP inhibitor	Hop
Betulin	Triterpene	SREBP inhibitor	Birch bark
Sodium palmitate	Satured fatty acid	SREBP inhibitor	Palmitic acid
β- Nicotinamide mononucleotide	NAD precursor nucleotide	SREBP activator	Amide of nicotinic acid

Neuronal quantification of LDs number

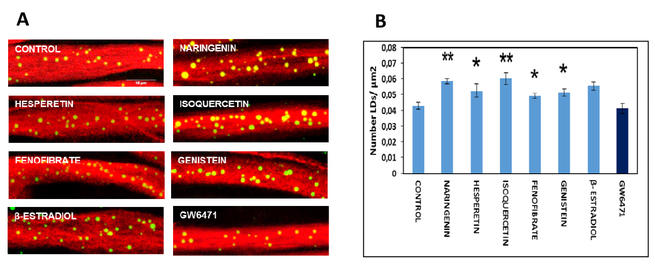


Fig.1 (A) Representative images of *Drosophila* larvae axons labeled with HRP red and LDs labeled with BODIPY 493/503. **(B).** Quantification of LDs number in axons after treatment with different PPARs modulators.
Scale bar, 10 µm. In all experiments significance was calculated using unpaired t-test (two tailed). Differences were considered statistically significant at p<0.05 (*) and p<0.005 (**).

Muscle quantification of LDs number

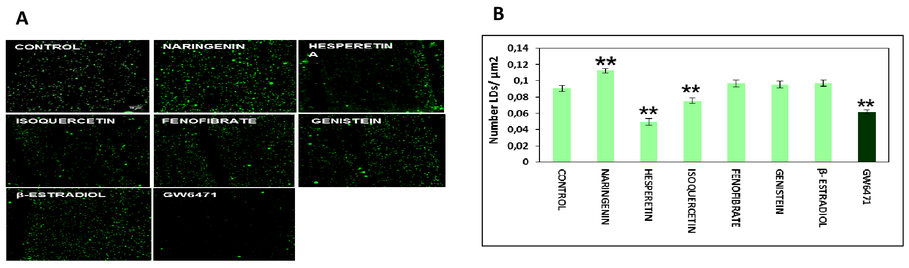


Fig.4 (A) Representative images of *Drosophila* larvae muscles labeled with HRP red and LDs labeled with BODIPY 493/503. **(B).** Quantification of LDs number in axons after treatment with different PPARs modulators.
Scale bar, 10 µm. In all experiments significance was calculated using unpaired t-test (two tailed). Differences were considered statistically significant at p<0.05 (*) and p<0.005 (**).

Relative gene expression

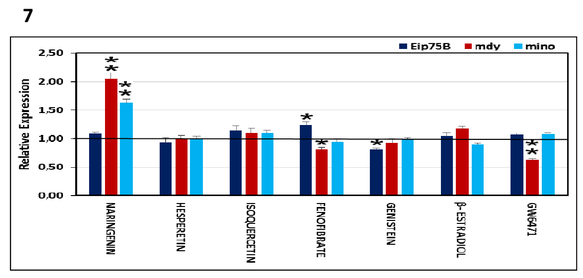


Fig.7. Relative gene expression of Eip75B, midway and minotaur after treatment with PPARs modulators. In all experiments significance was calculated using one-sample t-test. Differences were considered statistically significant at p<0.05 (*) and p<0.005 (**).

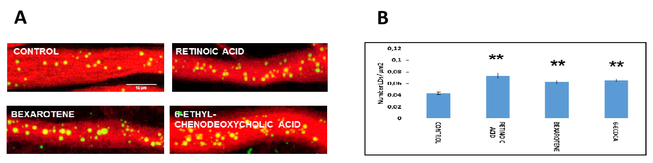


Fig.2.(A) Representative images of *Drosophila* larvae axons labeled with HRP red and LDs labeled with BODIPY 493/503. **(B).** Quantification of LDs number in axons after treatment with different RXR modulators.
Scale bar, 10 µm. In all experiments significance was calculated using unpaired t-test (two tailed). Differences were considered statistically significant at p<0.05 (*) and p<0.005 (**).

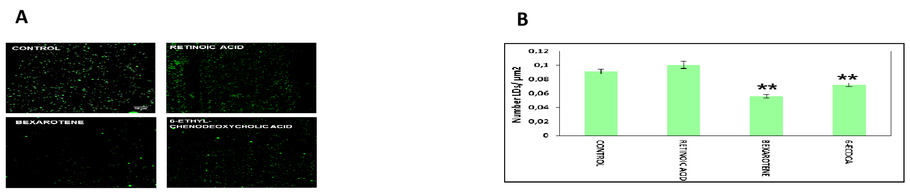


Fig.5 (A) Representative images of *Drosophila* larvae muscles labeled with HRP red and LDs labeled with BODIPY 493/503. **(B).** Quantification of LDs number in axons after treatment with different RXR modulators.
Scale bar, 10 µm. In all experiments significance was calculated using unpaired t-test (two tailed). Differences were considered statistically significant at p<0.05 (*) and p<0.005 (**).

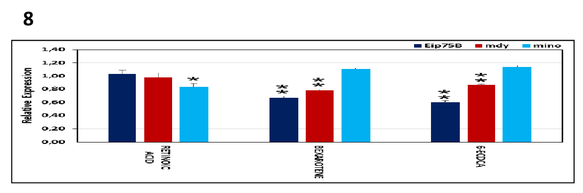


Fig.8 Relative gene expression of Eip75B, midway and minotaur after treatment with RXR modulators. In all experiments significance was calculated using one-sample t-test. Differences were considered statistically significant at p<0.05 (*) and p<0.005 (**).

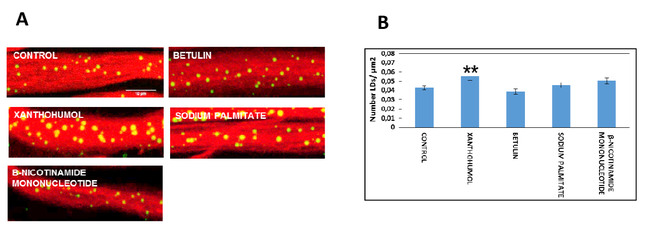


Fig.3 (.(A) Representative images of *Drosophila* larvae axons labeled with HRP red and LDs labeled with BODIPY 493/503. **(B).** Quantification of LDs number in axons after treatment with different SREBP modulators.
Scale bar, 10 µm. In all experiments significance was calculated using unpaired t-test (two tailed). Differences were considered statistically significant at p<0.05 (*) and p<0.005 (**).

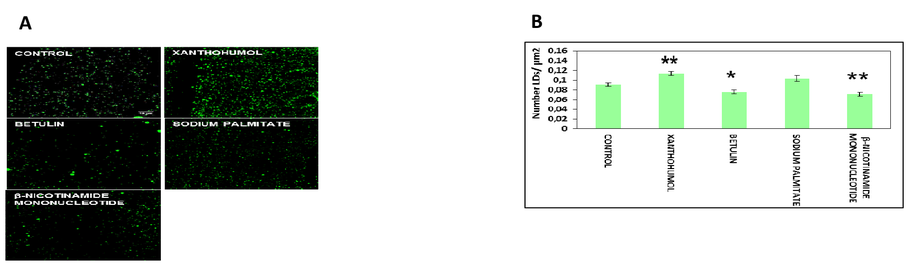


Fig.6 (A) Representative images of *Drosophila* larvae muscles labeled with HRP red and LDs labeled with BODIPY 493/503. **(B).** Quantification of LDs number in axons after treatment with different SREBP modulators.
Scale bar, 10 µm. In all experiments significance was calculated using unpaired t-test (two tailed). Differences were considered statistically significant at p<0.05 (*) and p<0.005 (**).

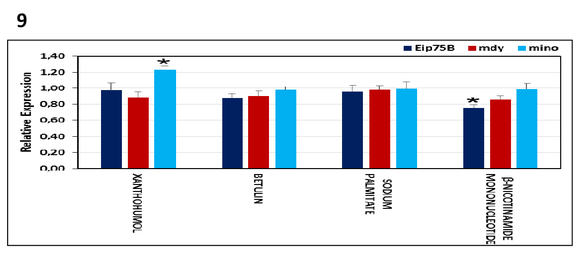
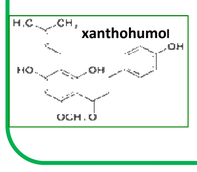
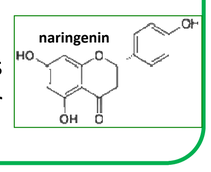


Fig.9. Relative gene expression of Eip75B, midway and minotaur after treatment with SREBP modulators. In all experiments significance was calculated using one-sample t-test. Differences were considered statistically significant at p<0.05 (*) and p<0.005 (**).

Conclusions



This study confirmed that *Drosophila* is a useful tool to test phytochemical effects *in vivo*.
Results showed that the tested compounds affect lipid metabolism and highlighted the strong positive modulation of naringenin and xanthohumol on lipid droplets biogenesis in muscles and axons. Therefore these two phytochemicals can be used to restore the lipid imbalance caused by pathologic conditions that block or impair LDs biogenesis/turnover.



1. Bier E, Bodmer R. Drosophila, an emerging model for cardiac disease. *Gene*. 2004;342(1):1-11.
2. Pandey UB, Nichols CD. Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. *Pharmacol Rev*. 2011;63:411-436.