Mitohometic responses in larvae fed 1:16 P:C food

PLOS Genetics

Wen Aw[1]

[1]z3314717@unsw.edu.au

dx.doi.org/10.17504/protocols.io.rtfd6jn

Cage Studies

Wen Aw

EXTERNAL LINK

https://doi.org/10.1371/journal.pgen.1007735

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Aw WC, Towarnicki SG, Melvin RG, Youngson NA, Garvin MR, Hu Y, Nielsen S, Thomas T, Pickford R, Bustamante S, Vila-Sanjurjo A, Smyth GK, Ballard JWO (2018) Genotype to phenotype: Diet-by-mitochondrial DNA haplotype interactions drive metabolic flexibility and organismal fitness. PLoS Genet 14(11): e1007735. doi: 10.1371/journal.pgen.1007735

PROTOCOL STATUS

Working

1 For inhibitors, freshly prepared aldose reductase (polyol pathway) inhibitor (Epalrestat, Sigma SML0527) and carnitine palmitoyltransferase-1 inhibitor (Etomoxir, Sigma E1905) were solubilised in water to prepare a 5 mM stock.

2 Glycolysis inhibitor (2-Deoxy-D-Glucose, Sigma D8375) was solubilised in water to prepare a 500mM stock.

3 The stock solutions were added to the 1:2 and 1:16 P:C diets to final concentrations of 25 ÂµM Epalrestat, 12.5 ÂµM Etomoxir and 5mM 2-Deoxy-D-Glucose.

4 Briefly, about 100, 3-4 d old, flies of each mitotype were released into population cages and allowed to lay eggs on Petri plates containing 4% agar and 10% treacle supplemented with yeast.

5 Eggs were collected, washed, and equivalent numbers placed onto bottles containing 1:2 or 1:16 P:C food with the inhibitors added.

6 Microbiome was added after 2 d. Flies eclosing in a 3 d window were collected and counted, and eclosion percentage was calculated.

7 To test specific hypotheses, we replaced sucrose as the dietary sugar. The 1:16 P:C diet was prepared without the addition of sucrose.

8 Then, 200 ml of food was combined with 1.868 g of either sucrose (Sigma S0389)Â as theÂ control, sorbitol (Sigma S1876), fructose (Sigma F0127), mannose (Sigma M6020), fucose (Sigma F2252), xylose (Sigma X3877) or gluconate (Sigma G1951). Each new diet was poured into 8 bottles.

9 Equal amounts of eggs harbouring Alstonville or Dahomey mtDNA were added to each diet and microbiome was added after 2 d. Flies

were kept at 23Â° C, 50% humidity on a 12 h light/dark cycle.

10 Emerging adult female flies were counted over 3 d, and percentage eclosion of each mitotype was recorded.Â