Brassica Glucosinolates Improve Metabolism Efficiency of Drosophila Melanogaster by Promoting Advantageous Mitochondrial Respiration

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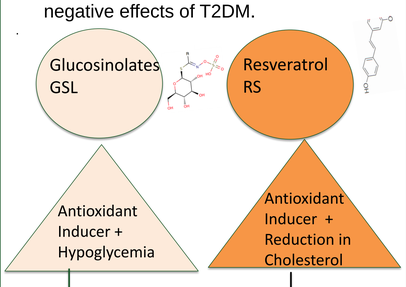
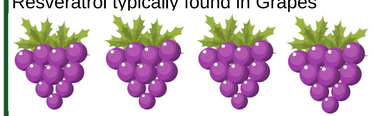
Background

- **Obesity** is the largest contributor to the development of Type II Diabetes Mellitus **(T2DM)** in the USA.
- **T2DM** is a compounding problem in children due to vascular damage, lifespan reductions and medication tolerance over time. Furthermore, the rates of T2DM will rise form 171 million to 366 million by 2030 (Sholeson, Herroro, Naaz, 2007).
- The compilation of lifespan alterations, pathology and deteriorating metabolic processes necessitates an investigation to find compounds which limit the negative effects of T2DM.



- Glucosinolates are typically found in Brassica oleracea.

Resveratrol typically found in Grapes



Why Fruit Flies?

- 75% similarity to humans with respect to disease
- Simple and Sequenced Genome
- Understood Gene Pathways for Hyperglycemia, Inflammation, Metabolism and Longevity

Why High Fat?

- Drosophila on high fat diet of 30 percent or more typically display reductions in lifespan, increased oxidative stress, and signs of hyperglycemia similar to T2DM in humans. (Birse, et. al., 2010

Question

What effect do resveratrol, glucosinolates, and brassica extracts have on lifespan, gene expression, and metabolism of fruit flies predisposed to hyperglycemia?

Hypothesis

If Resveratrol and Glucosinolates are supplemented in an animal's diet, then their metabolism will have an improved capacity to uptake glucose from the bloodstream.

Methods

Study of Metabolic Efficiency (OCR, ECAR)

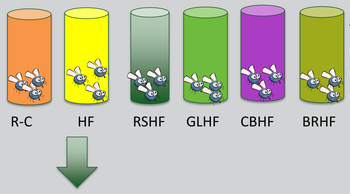


fig: displays drosophila larvae (n=3) raised on different diets.

Fig: displays live drosophila larvae from each diet placed in triplicates in XF media in 24 well plates

fig: Displays Seahorse XF 24 Flux Analyzer

Relevancy: Seahorse measures mitochondrial respiration and glycolysis in-vivo Thus, indicating mitochondrial stress of flies.

Lifespan Analysis of Fruit Flies on Different Supplements

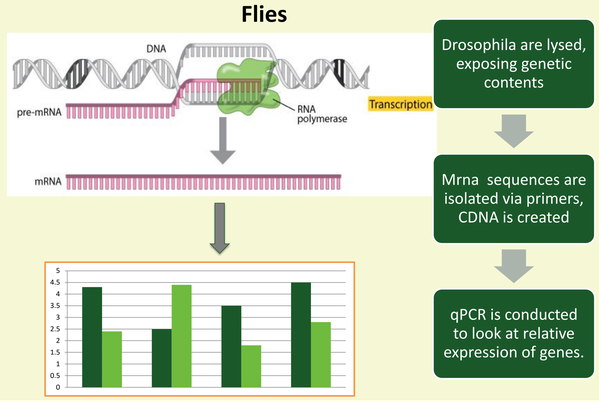
Fruit fly larvae are separated into 6 Dietary Groups R-C, HF, RSHF, GLHF, BRHF, CBHF

Dead fruit flies are removed from each group.

Dead fruit flies are then placed in -80C freezer and statistical analysis is conducted

- **R-C**: Regular Control
- **HF:** High Fat Control
- **RSHF:** Resveratrol High Fat
- **GLHF:** Glucoraphanin High Fat
- **BRHF:** Broccoli High Fat
- **CBHF:** Cabbage High Fat

QPCR of Advantageous Gene Expression for Fruit Flies



Gene	Function	Primer Sequence Forward	Primer Sequence Reverse
Insulin Signaling and Gluconeogenesis			
IGF II	Regulation of mRNA translation	ACAACAG GCAACAC ACCCAT	ACATCAAG ATTTCAGG CTAGTACG
Jun N Kinase	Regulation of stress response and cellular morphology	GTGCCGC TAAACAC TTTGCT	AAGCGGC GCATACTA TTCCT
DILP 2	Nutrient based growth stimulator	AGGTGCT GAGTATG GTGTGC	TGTCGGCA CCGGGCAT
DILP 3	Hormonal; insulin binding activity	CTTATGAT CGGCGGT GTCCA	CACGGGGT CCAAAGTT CTCT
PEPCK	Binding of GTP	GCTGGAC GAGCTAT CTTCCC	TGATGGGG TCAGTACG GGAT
Fructose-1,6-bisphospatase	Gluconeogenesis	TGTCGAT TTGTTAG GCCAGC	TGGAGAGC ACGTCCAG TTTC
FOXO Insulin dependent Transcription factor			
TAG (CG8093)	lipase activator and hydrolase activity	GCATATAA AAGGCAC GGGCG	TCCCCTAA CAATGGTG ACAGTT
Pantothenate Kinase	Binding ATP	TGTTGAG TGTGCGT GGAACT	ATTGTCCA TCTGTAGG TGCGT
Acyl CoA Dehydrogenase (CG9547)	metabolism of trptophan, hydroxlysine catabolism, oxidation and reduction, lysine catabolism	GGACTCC GCTTATC GGTCTG	AAAGGCAC CAATCAGC TTGC
SIRTUIN 1	inhibition of macromoleucle metabolism, and activation of insulin uptake by cells	CTCATCC GTGGAAC CCGAC	GGCAGTCC CTCGCCGA AAA
SIRTUIN 2	lifespan determination	AAGGAGG AACAACC GACGAC	CCGGAATG CCAGCAGA TGTA
MTH	G protein coupled receptor & lifespan determination	ACAACCG CGAAGAA CAGGAT	GGATGGTT CCTCTCCT GCAA
Cap N Collar Nrf2	Modulates Gene Activity in response to oxidative stress	GTTTTCA AGCTCAC CACCAAT	TCGATACG TGAGCGAA TGGG
Housekeeping gene			
RpL32	Ribosomal protein	GGCAAGC TTCAAGA TGACCA	GTTCGATC CGTAACCG ATGT

Results

Study of Metabolic Efficiency (OCR, ECAR)

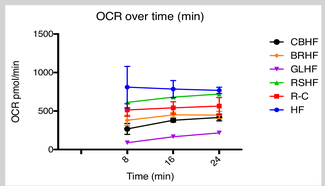


fig: Displays oxygen consumption rate measured in pmol/min over 24 min period. This reading indicates mitochondrial respiration. Readings are calculated every 8 minutes. First data point is excluded for calibration, and triplicate is analyzed.

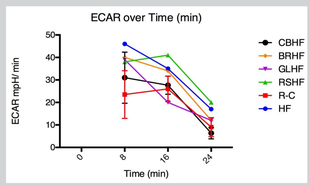


fig: Depicts ECAR variation in mpH/min over 24 minutes. Readings are calculated every 8 minutes. First data point is excluded for calibration, and triplicate is analyzed. No statistical significance was detected in rate of glycolysis between varying groups.

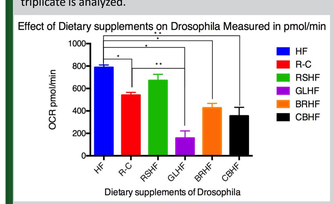


fig: Displays mean effect of dietary supplements on Drosophila measured in pmol/min. Significance indicated between HF and RC (p< 0.0217), HF and GLHF (p< 0.0011), HF vs BRHF (p< 0.0041), HF and CBHF (p< 0.0027), R-C and GLHF (p< 0.0093). Oxygen consumption in drosophila appears to be the most elevated in high fat (HF) compared to rest of drosophila diet.

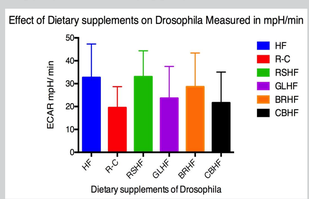


fig: Displays the mean concentrations of ECAR in mpH/min per dietary group of Drosophila melanogaster. No statistical significance was detected in rate of glycolysis between varying groups.

Lifespan Analysis of Fruit Flies on Different Supplements

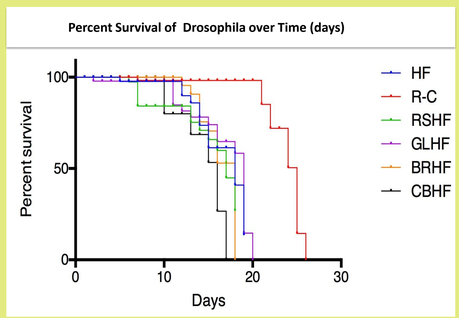


fig: displays Kaplan Meier curve (p value < 0.05). Statistically significant differences were indicated between Regular diet (R-C) and high fat diet (HF) p<0.0001. No statistical significance was indicated between differing supplement groups on high fat diet (RSHF, GLHF, BRHF,CBHF) when compared to HF control.

QPCR of Gene Expression for Fruit Flies

fig displays relative gene expression. No statistical significance indicated for IGF2.

fig displays relative gene expression. No statistical significance indicated for TAG.

fig displays relative gene expression. No statistical significance indicated for SIRT1.

fig displays relative gene expression. No statistical significance indicated for DILP2.

Conclusions

Study of Metabolic Efficiency (OCR, ECAR)

- High fat Drosophila exhibit increased metabolic stress via OCR when compared to Regular Diet, which is consistent with OCR results in humans with T2DM vs normal humans.
- Fruit flies on High fat diet supplemented with (GL, BR, CB) exhibited a higher mitochondrial efficiency relating to oxygen consumption when compared to High Fat Control Drosophila.

Lifespan Analysis of Fruit Flies on Different Supplements

- Longevity: Lifespan was reduced in Drosophila fed High fat diet compared to Regular diet, which is consistent with data on lifelong diabetic longevity. (Hartman, et. al., 2014)
- No significance was found in lifespan High fat fed control vs High fat fed fruit flies (GL, RS, CB, BR).

Gene Expression Study

- Dietary supplementation of Glucosinolates derived from Brassica Crops, and Resveratrol have no statistically significant impact on gene expression for fruit flies.
- Primary advantageous effect of Glucosinolates comes from ability to oxygen consumption rate of mitochondria of fruit flies.

Future Directions

- Supplement Mice with GSL's
- Supplement Mice with Resveratrol
- Investigate gene expression similarities between Alzheimer's and Diabetes.
- Investigate Mitochondrial efficiency in Mice

References

- Birse RT, Choi J, Reardon K, Rodriguez J, Graham S, Diop S, et al. High-fat-diet-induced obesity and heart dysfunction are regulated by the TOR pathway in Drosophila. Cell Metab. 2010; 12(5):533–544. [PubMed: 21035763]
- *DNA to RNA*, by unknown, Nature, 2015, accessed July 2018.
- Hartman, M.L., Shirihai, O.S., Holbrook, M., Xu, G., Kocherla, M., Shah, A., Fetterman, J.L., Kluge, M.A., Frame, A.A., Hamburg, N.M. and Vita, J.A., 2014. Relation of mitochondrial oxygen consumption in peripheral blood mononuclear cells to vascular function in type 2 diabetes mellitus. *Vascular medicine*, *19*(1), pp.67-74.n
- Seahorse XF 24 Flux Analyzer, by unknown. ECU, 2018, accessed July 2018, http://www.ecu.edu/cs-dhs/ecdoi/seahorse.cfm
- Shoelson, S.E., Herrero, L. and Naaz, A., 2007. Obesity, inflammation, and insulin resistance. *Gastroenterology*, *132*(6), pp.2169-2180.