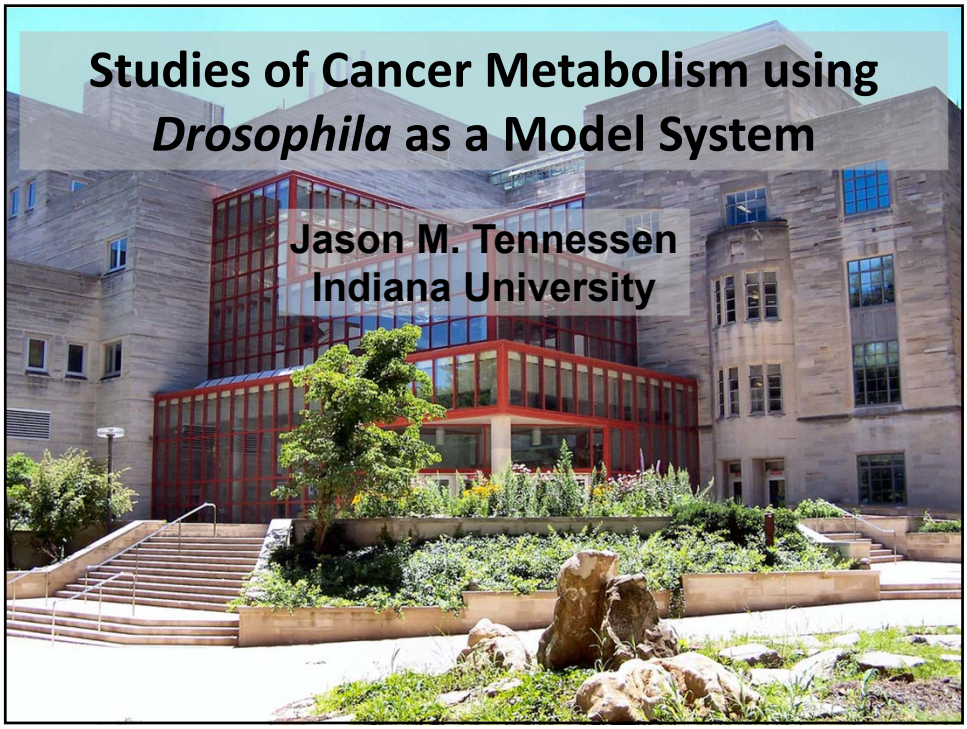
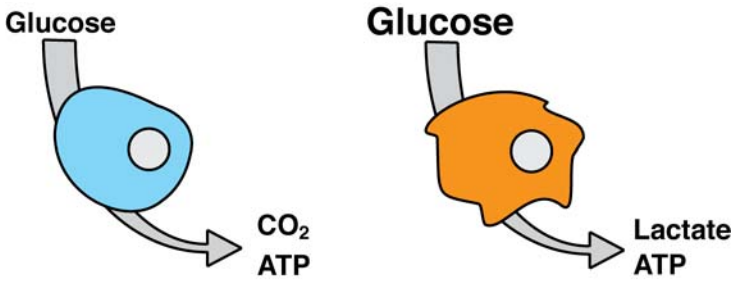
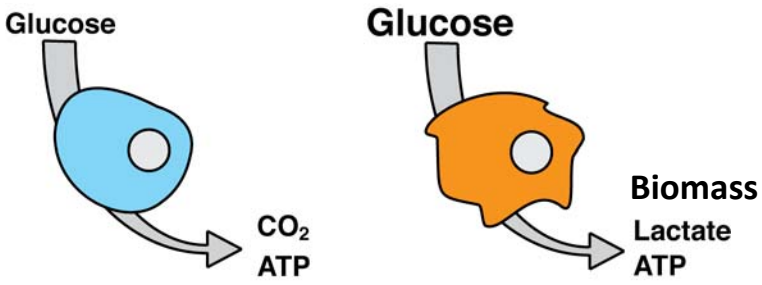


Tumor cells use aerobic glycolysis to generate energy



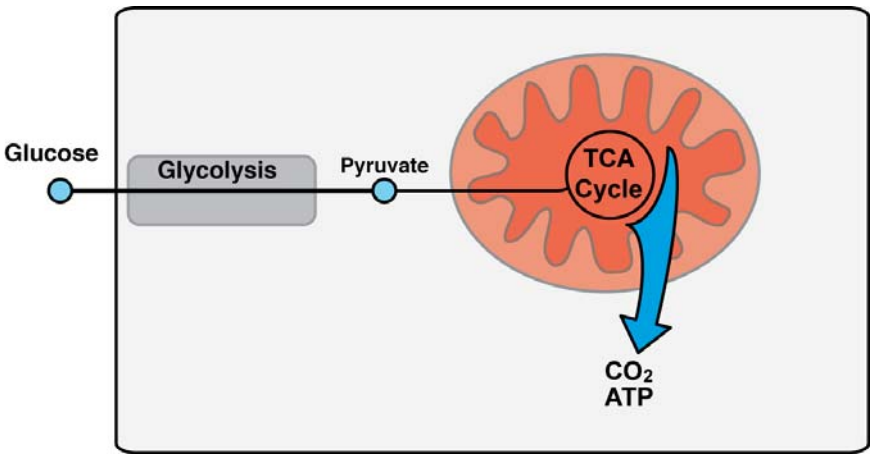
Quiescent Cell Tumor Cell

Tumor cells use aerobic glycolysis to generate energy and biomass

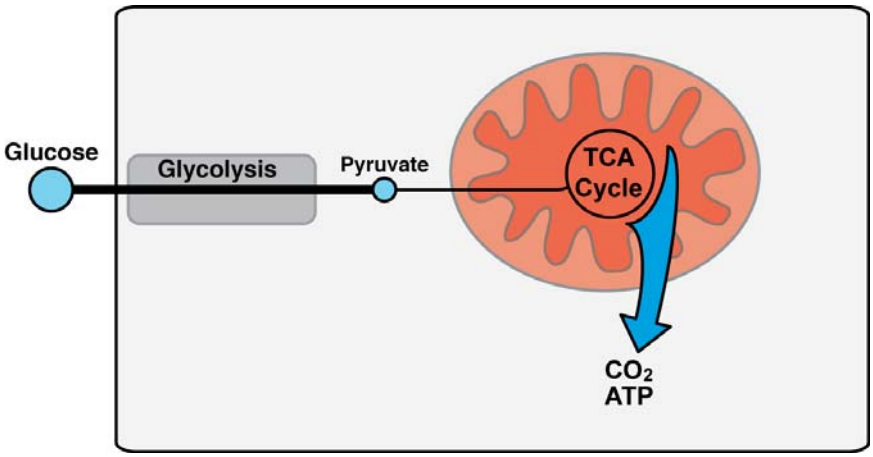


Quiescent Cell Tumor Cell

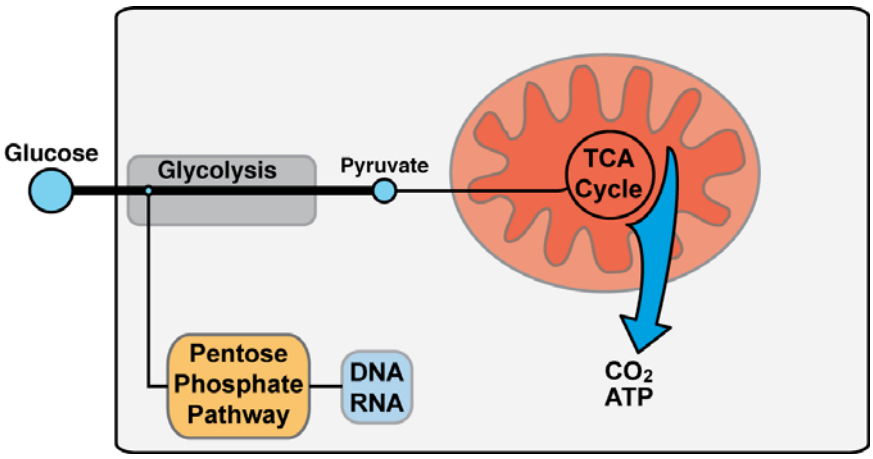
Glycolysis fuels oxidative metabolism



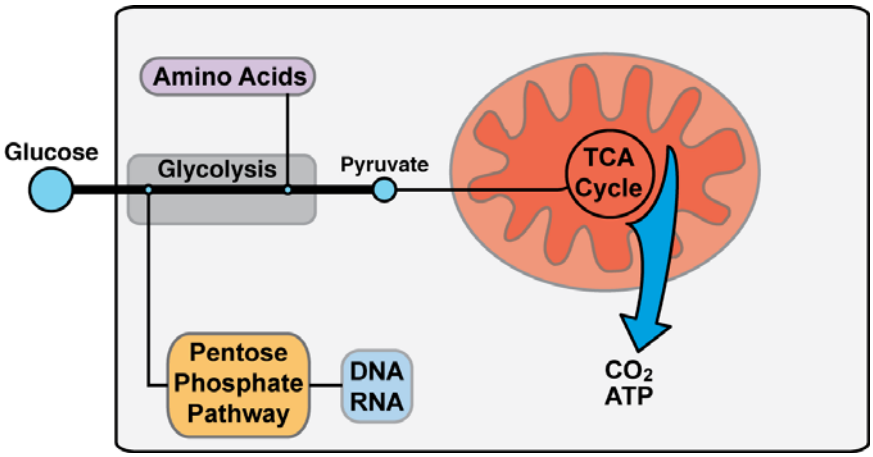
Aerobic glycolysis supports biosynthesis



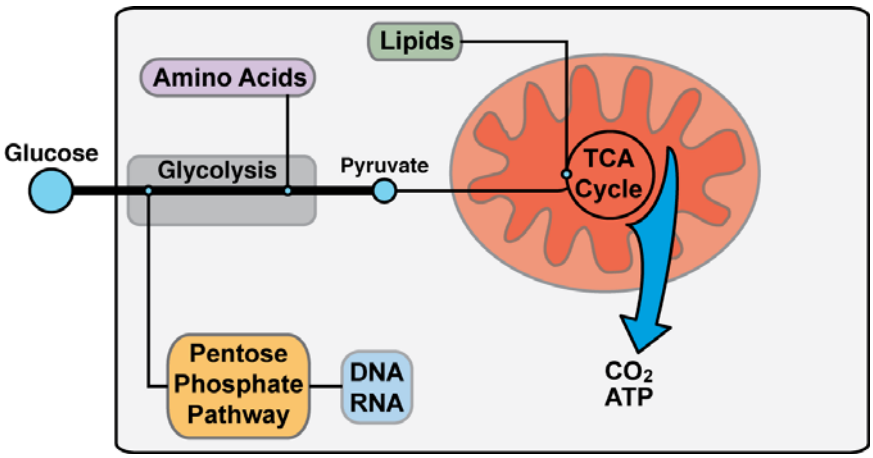
Aerobic glycolysis supports biosynthesis



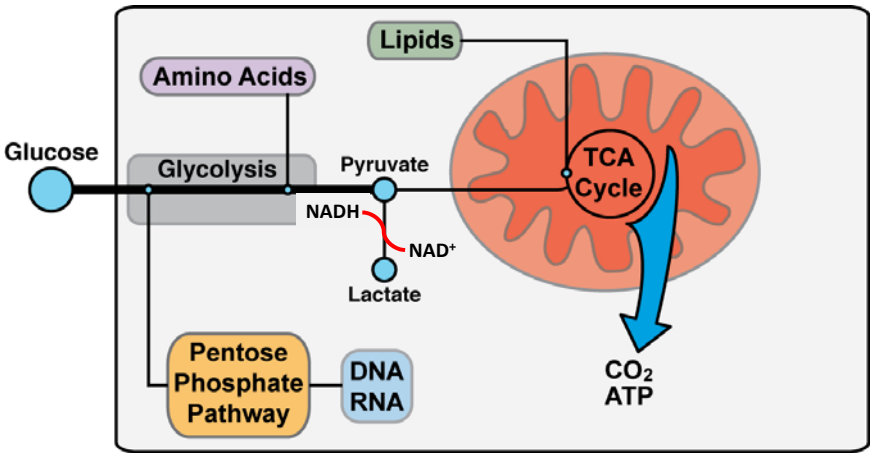
Aerobic glycolysis supports biosynthesis



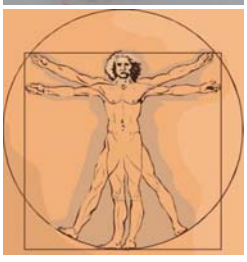
Aerobic glycolysis supports biosynthesis



Aerobic glycolysis supports biosynthesis

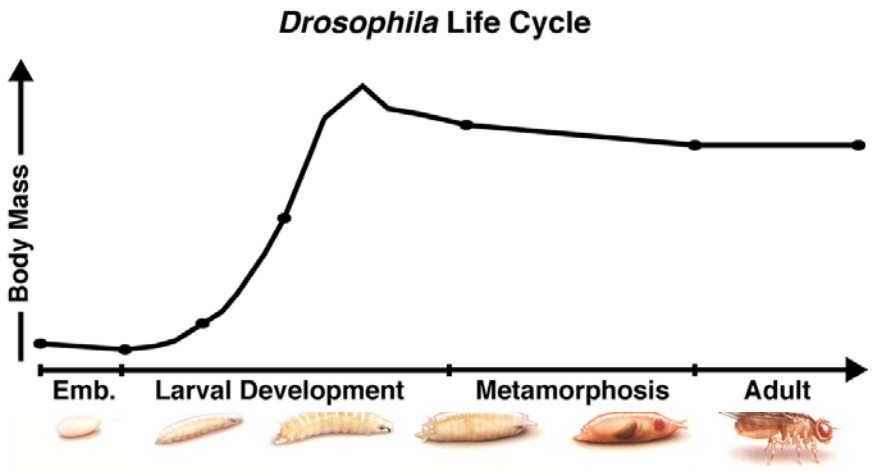


Studies in *Drosophila* predict metabolic gene function in humans

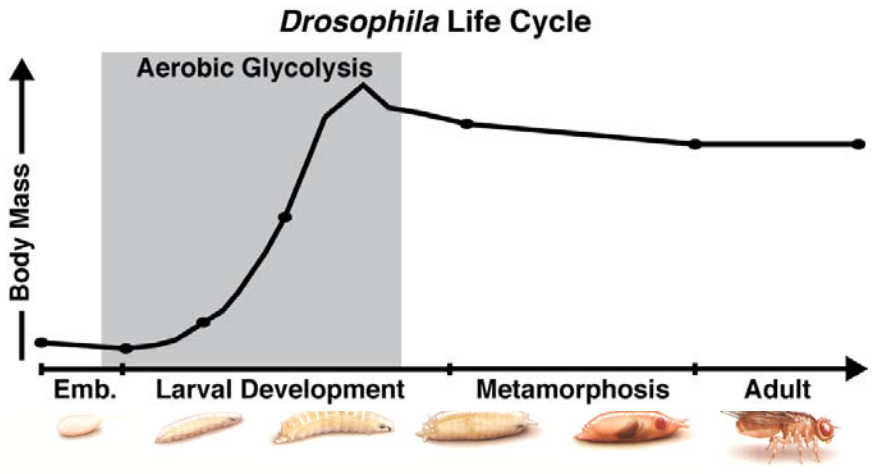


- Metabolic regulators such as insulin, Tor, and myc are conserved in flies
- Analogous tissues regulate systemic metabolic processes
- Used to model diabetes, obesity, and heart failure
- Studies in *Drosophila* have predicted the function of mammalian homologs

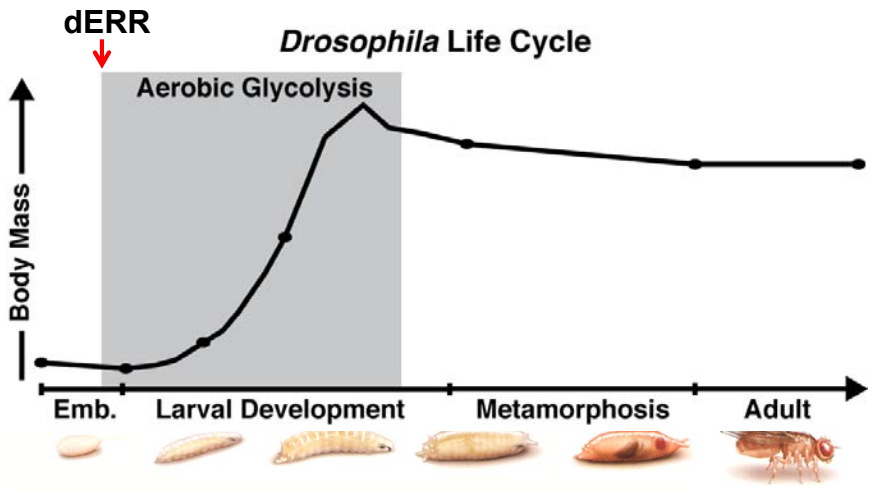
Drosophila larvae undergo rapid growth



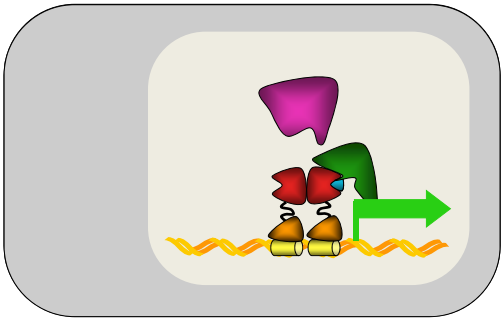
Aerobic glycolysis supports *Drosophila* larval growth



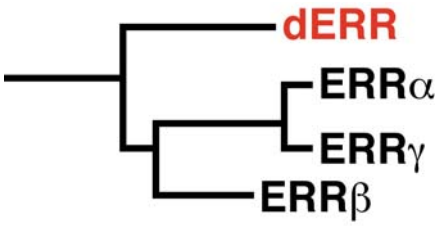
The *Drosophila* Estrogen-Related Receptor promotes aerobic glycolysis



Nuclear receptors are key metabolic regulators

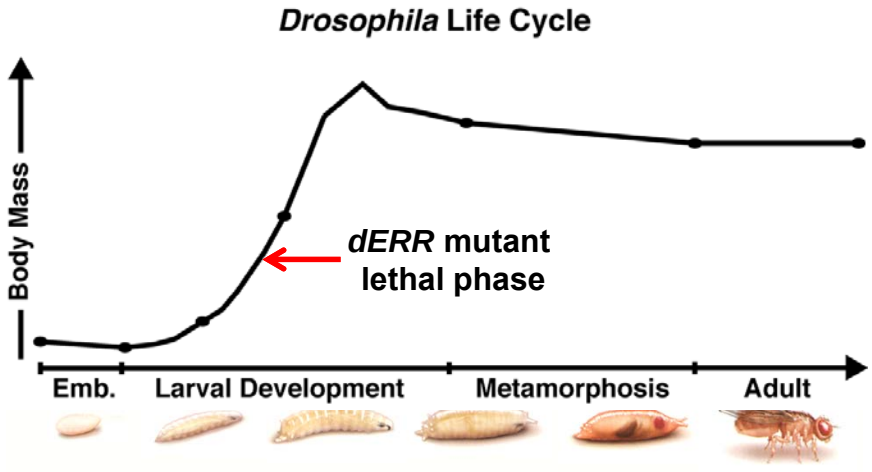


Estrogen-Related Receptors regulate metabolic function

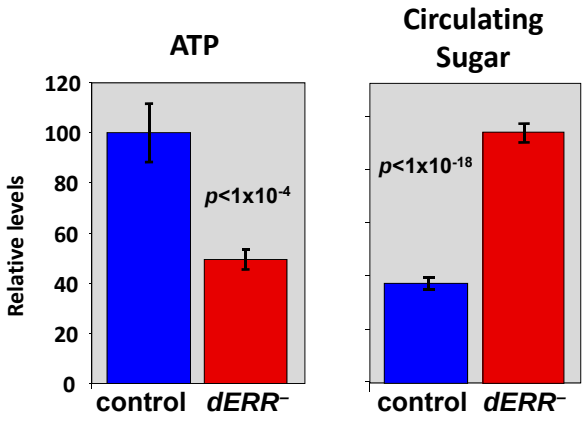


- Mammalian ERRs are key metabolic regulators
- Promote fat metabolism and mitochondrial biogenesis
- Functional redundancy limits efficacy of genetic studies

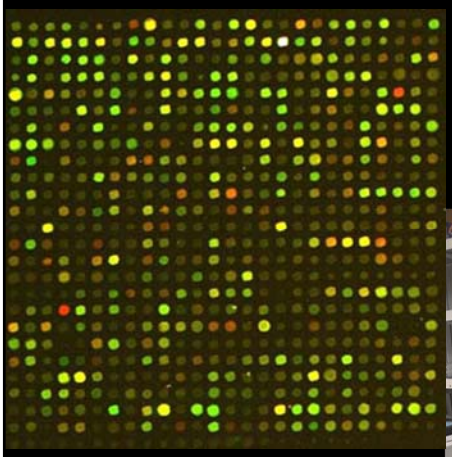
dERR mutants die during larval development



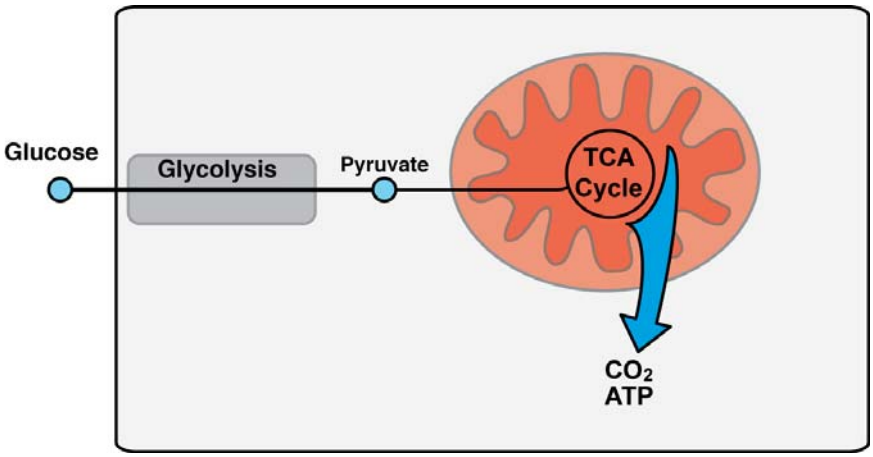
dERR mutants are energy starved and hyperglycemic



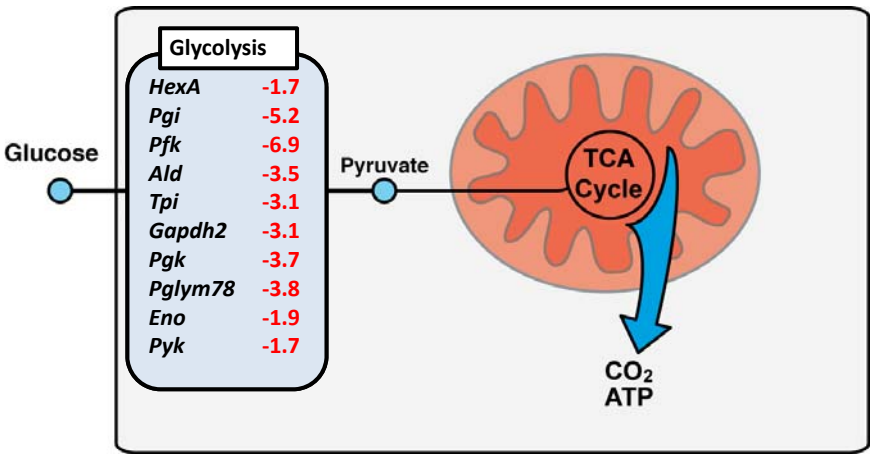
Genomic and metabolomic analysis of *dERR* mutants



dERR mutants do not use sugar to generate energy



dERR mutants display reduced expression of genes in the glycolytic pathway



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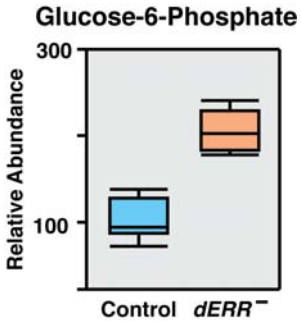

Glycolysis

HexA	-1.7
Pgi	-5.2
Pfk	-6.9
Ald	-3.5
Tpi	-3.1
Gapdh2	-3.1
Pgk	-3.7
Pglym78	-3.8
Eno	-1.9
Pyk	-1.7

dERR directly regulates genes in the glycolytic pathway

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Eno	-1.9
Pyk	-1.7

Predicted enzyme	Gene	Fold-Change	ERR binding site(s)
Hexokinase	*CG3001 (HexA)*	-1.7	-913 aGAAGGTCA -128 TGAAGGTCA
Phosphoglucose isomerase	*CG8251 (Pgi)*	-5.2	+871 gtAAGGTCA +908 ctgAGGTCA +1560 TGAAGGTCA
Phosphofructokinase	*CG4001 (Pfk)*	-6.9	+1420 TGAAGGTCA
Aldolase	*CG6058 (Ald)*	-3.5	-636 aGgAGGTCA +541 catAGGTCA +852 cCAAcGTCA
Triosephosphateisomerase	*CG2171 (Tpi)*	-3.1	-678 cGAAGGTCA
Glyceraldehyde phosphate dehydrogenase	*CG8893 (Gapdh2)*	-3.1	-847 aaAAGGTCA -489 TagAGGTCA -192 TatAGGTCA
Phosphoglycerate kinase	*CG3127 (Pgk)*	-3.7	+16 gCAAGGTCA
Phosphoglycerate mutase	*CG1721 (Pglym78)*	-3.8	-209 aaAAGGTCA +103 TgtAGGTCA +448 aCtAGGTCA
Enolase	*CG17654 (Eno)*	-1.9	+1450 cgAAGGTCA +2316 aTaAGGTCA +2334 TagAGGTCA
Pyruvate kinase	*CG7070 (Pyk)*	-1.7	-434 gtcAGGTCA +1002 TCAAGGTCA +2234 cGAAGGTCA

dERR binds to a canonical site in *Pfk*

Glycolysis	
HexA	-1.7
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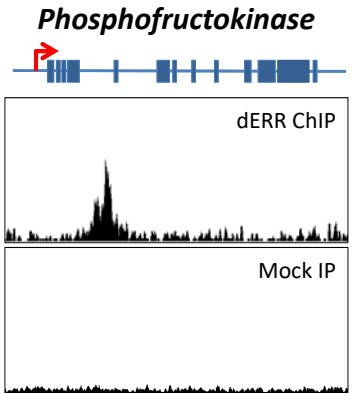

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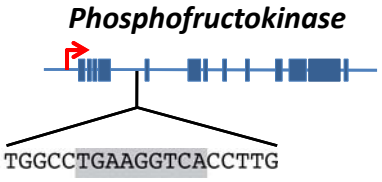
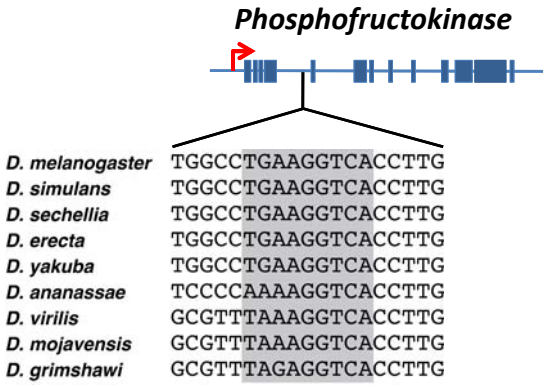
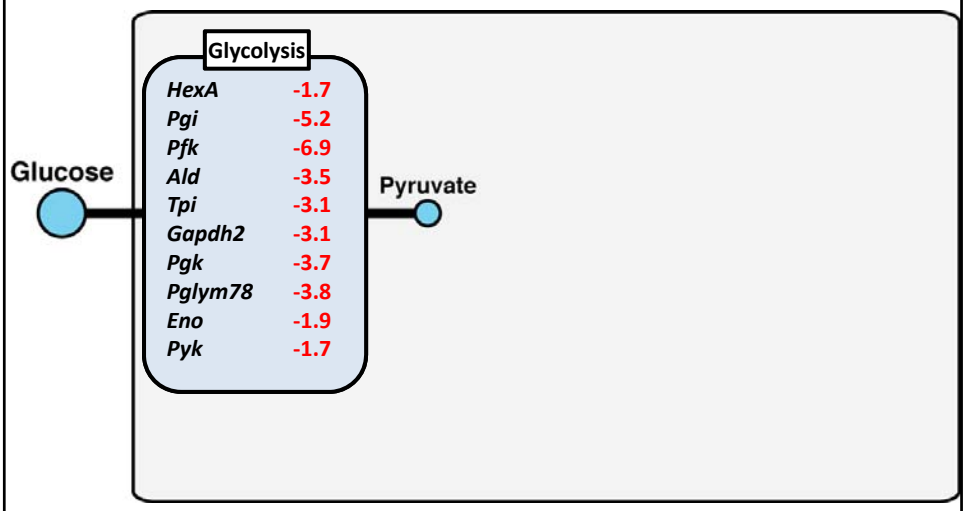


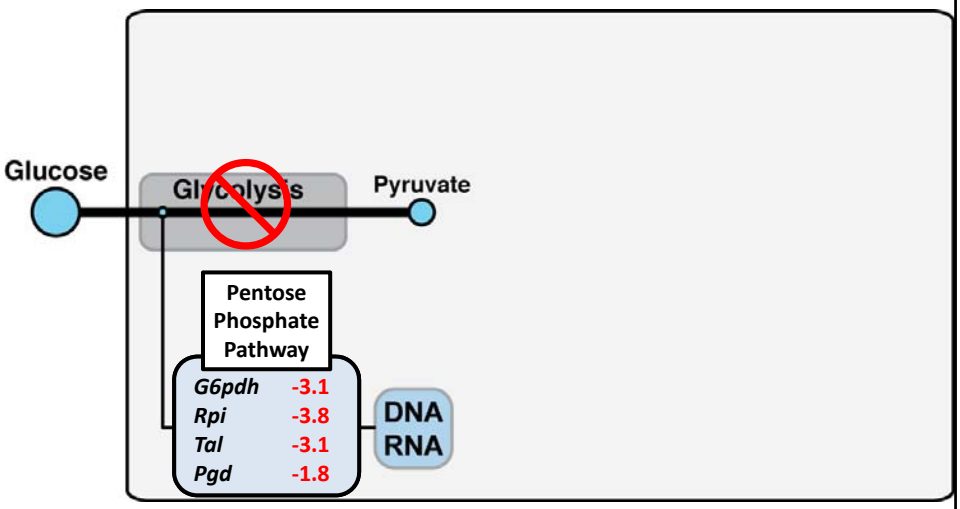
The dERR binding site in *Pfk* is conserved across species



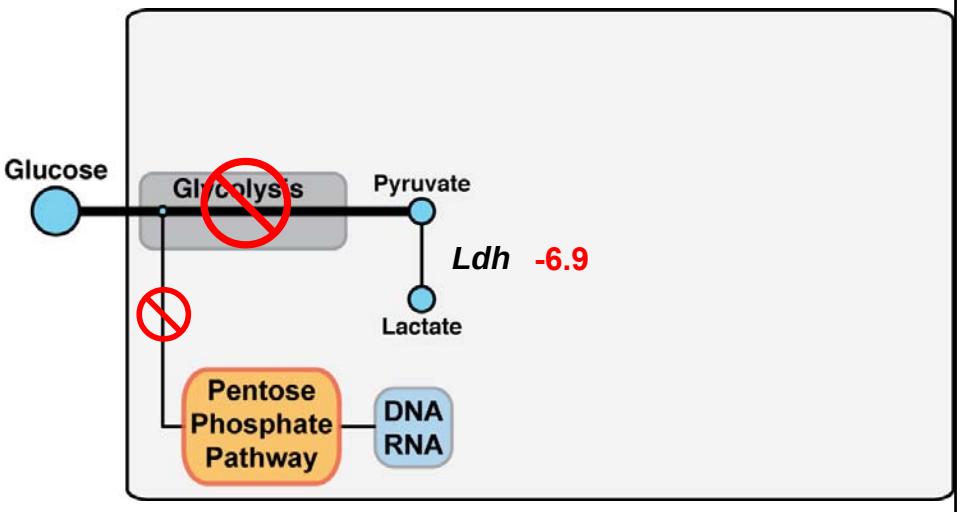
dERR mutants display reduced expression of genes in the glycolytic pathway



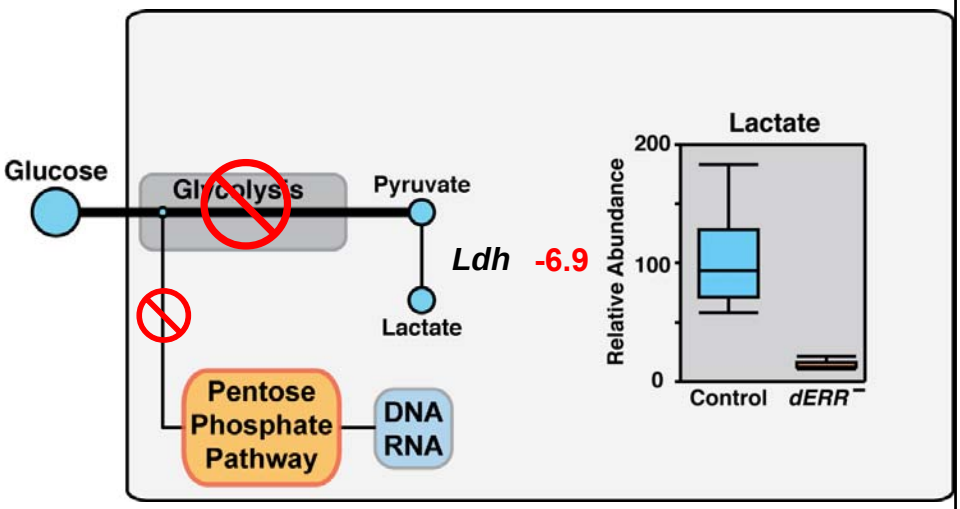
dERR is required for normal Pentose Phosphate Pathway Expression



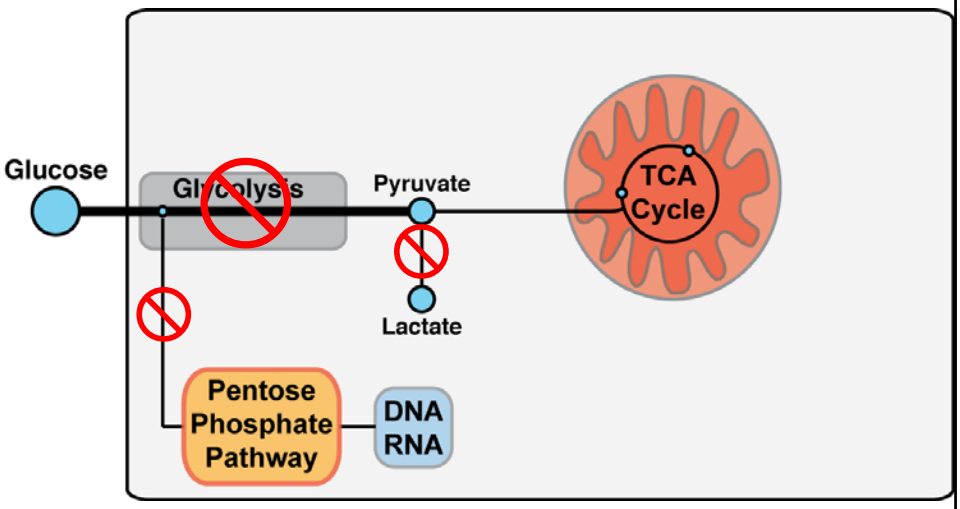
Lactate production is impaired in *dERR* mutants



Lactate production is impaired in *dERR* mutants



Proline is depleted in *dERR* mutants



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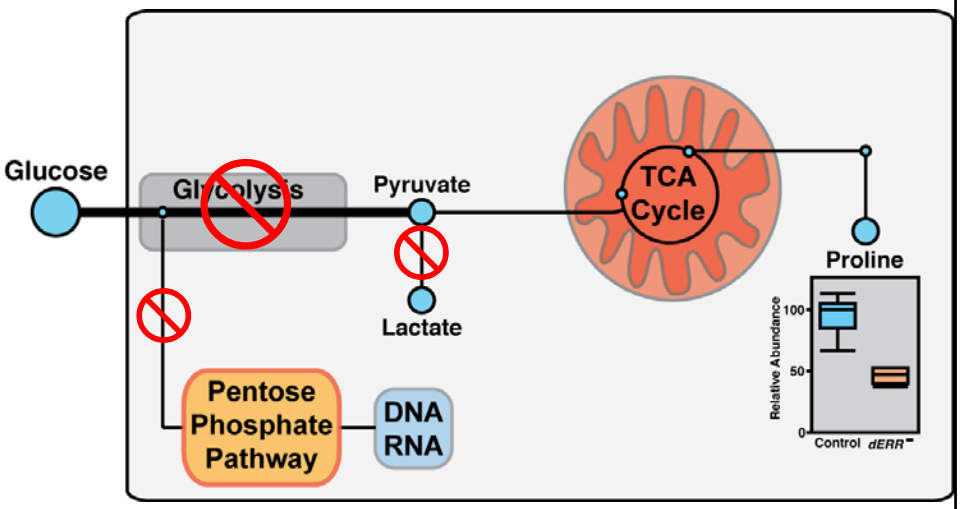
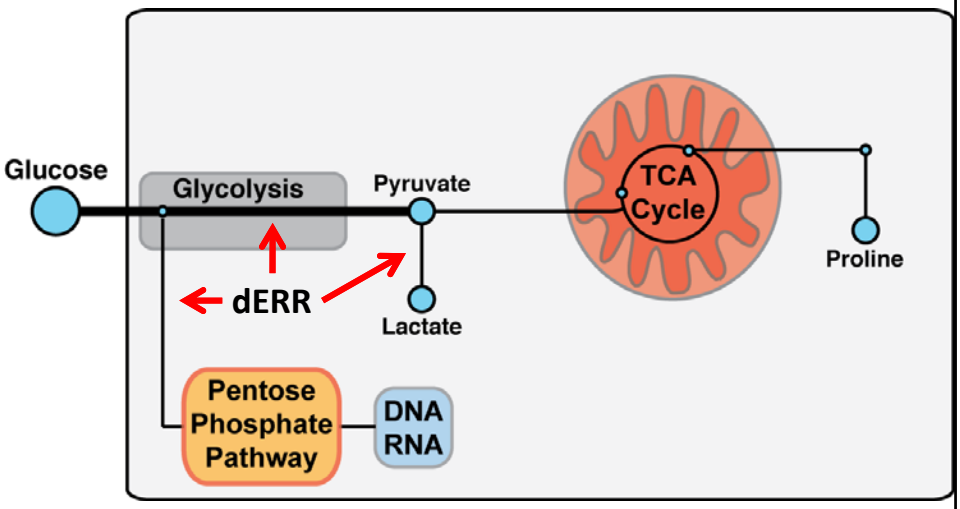


dERR mutant phenotypes suggest that *Drosophila* larvae use aerobic glycolysis



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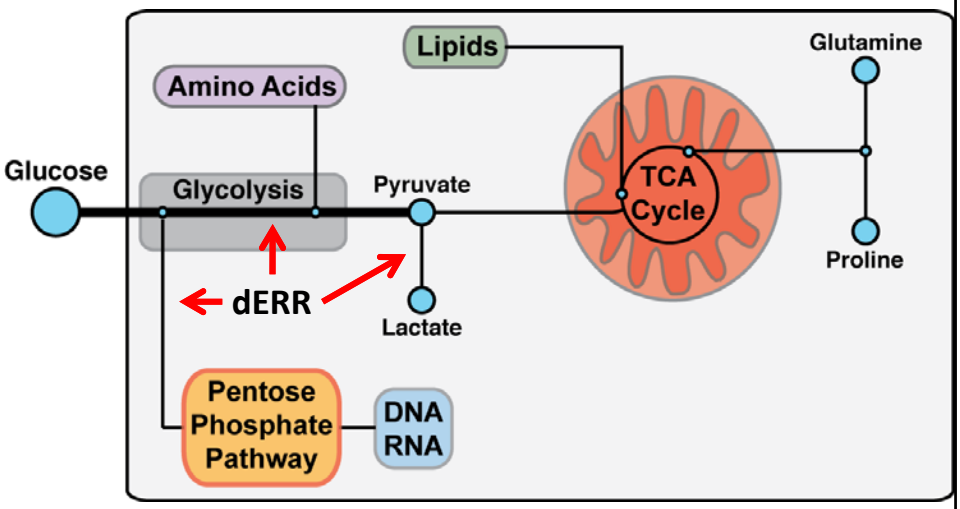
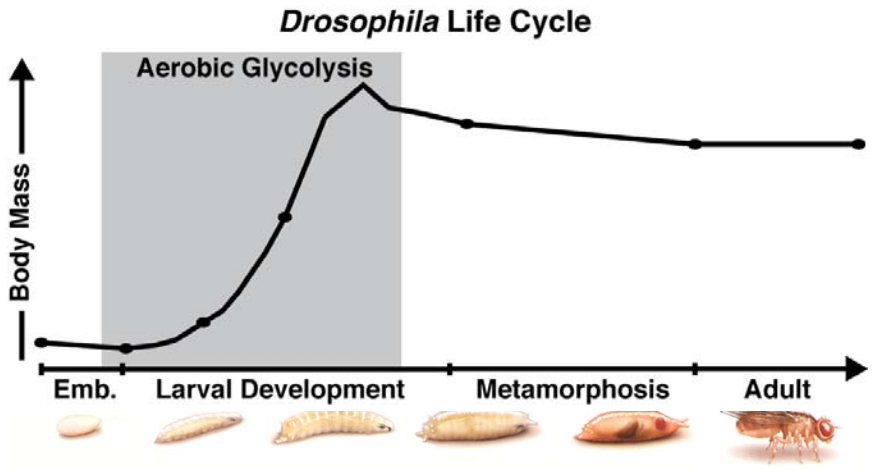
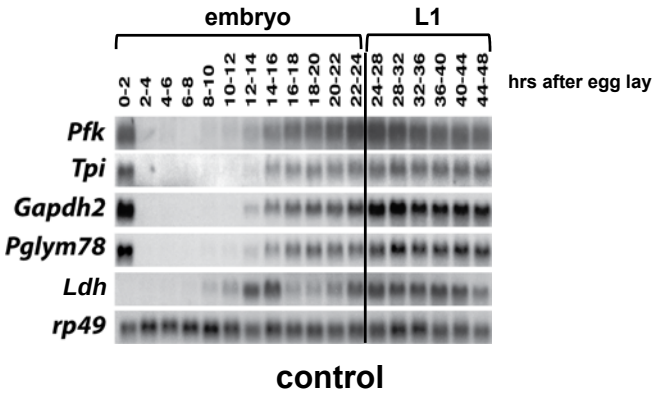


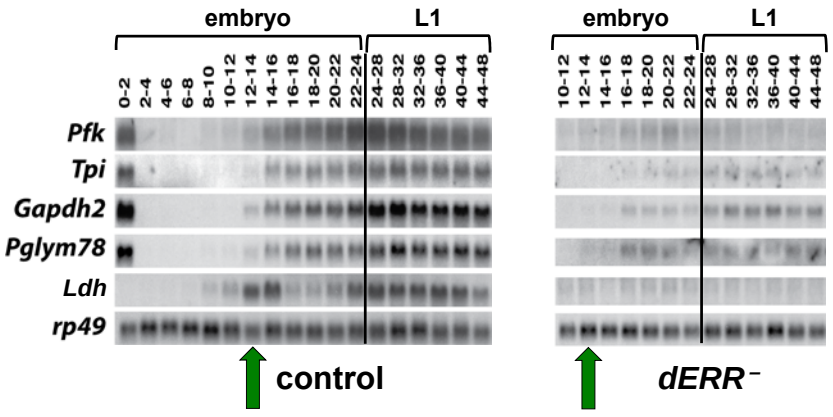
Drosophila increase ~200-fold in mass during larval development



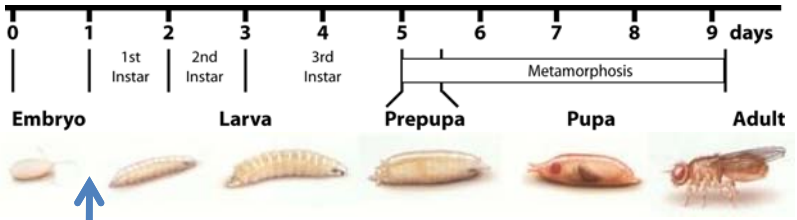
Genes that encode glycolytic enzymes are coordinately induced during embryogenesis



The glycolytic transcriptional program is not properly induced in *dERR* mutants



Conclusions



dERR

1. dERR establishes the metabolic state that supports juvenile growth
2. dERR directs a metabolic program related to the Warburg Effect during normal development
3. Mammalian ERR may contribute to cancer progression

Human ERRs promote the Warburg Effect

Oncogene (2012), 1–8

www.nature.com/onc

ORIGINAL ARTICLE

Regulation of glycolysis and the Warburg effect by estrogen-related receptors

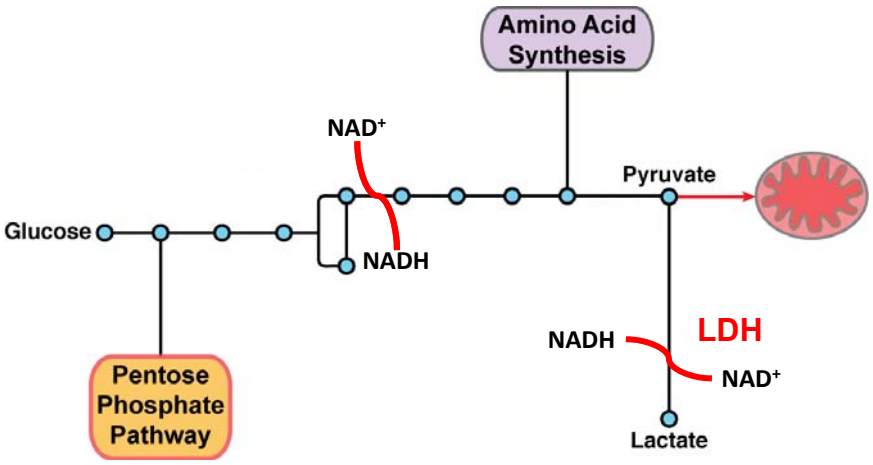
Q Cai, T Lin, S Kamarajugadda and J Lu

Cancer cells typically display altered glucose metabolism characterized by a preference of aerobic glycolysis, known as the Warburg effect, which facilitates cell proliferation. Hypoxia-inducible factor (HIF) and oncoprotein Myc are two prominent transcription factors that drive glycolysis. Previously, we reported that the estrogen-related receptors (ERRs) act as cofactors of HIF and enhance HIF-dependent transcription of glycolytic genes under hypoxia. ERRs are orphan nuclear receptors and key regulators of energy metabolism by orchestrating mitochondrial biogenesis, fatty acid oxidation (FAO) and oxidative phosphorylation. Here, we show that ERRs also stimulate glycolysis under normoxia. ERRs directly bind to and activate promoters of many genes encoding glycolytic enzymes, and the ERR-binding sites in such promoters are essential for ERR-mediated transcriptional activation. ERRs interact with Myc, and the two factors synergistically activate transcription of glycolytic genes. Furthermore, overexpression of ERRs increases glycolytic gene expression and lactate production. Conversely, depletion of ERRs in cancer cells reduces expression of glycolytic genes and glucose uptake, resulting in decreased aerobic glycolysis and cell growth. Taken together, these results suggest that ERRs are important transcriptional activators of the glycolytic pathway and contribute to the Warburg effect in cancer cells.

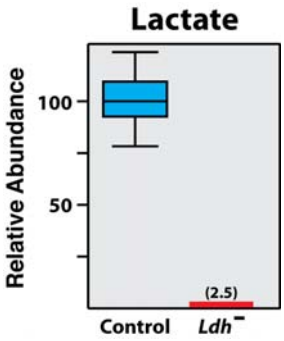
Oncogene advance online publication, 4 June 2012; doi:10.1038/onc.2012.221

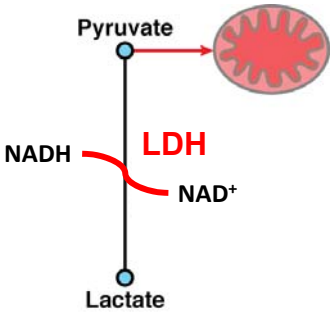
Keywords: aerobic glycolysis; Warburg effect; nuclear receptor; Randle cycle

What is the role of LDH is promoting larval growth?

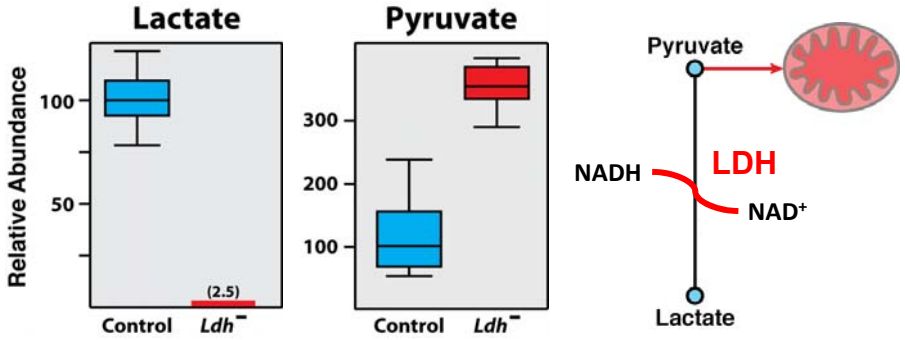


Ldh mutants fail to produce lactate

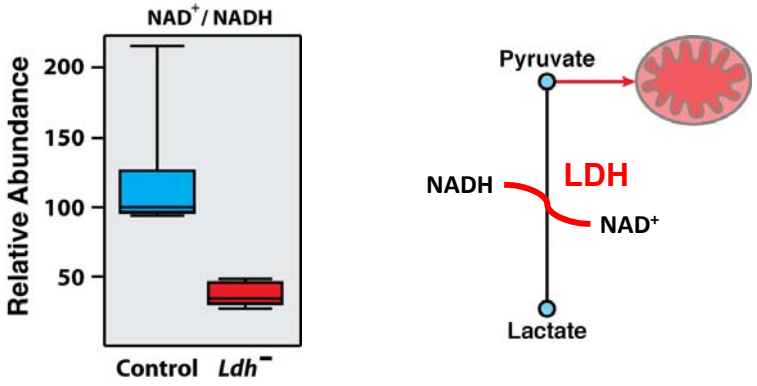




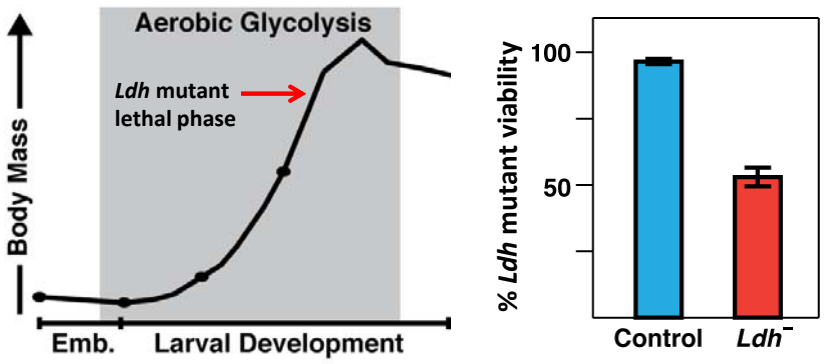
Pyruvate levels are elevated in *Ldh* mutants



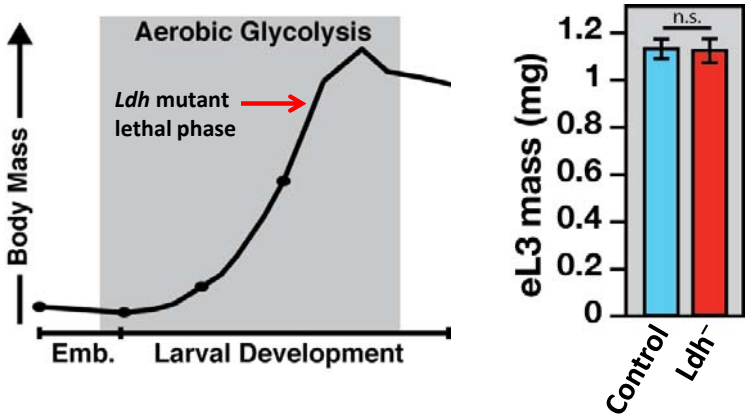
Ldh mutants fail to regenerate NAD⁺



Ldh mutants die near the end of larval growth



Ldh mutants grow at a normal rate



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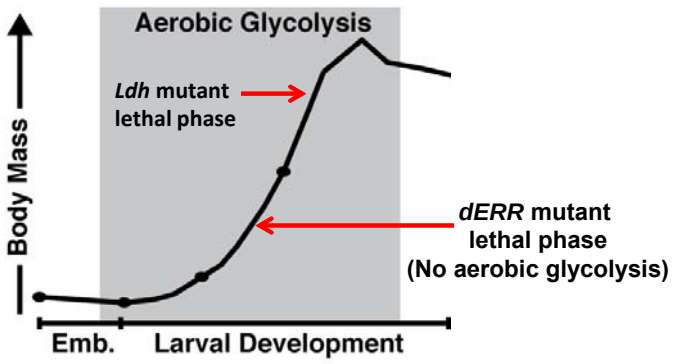
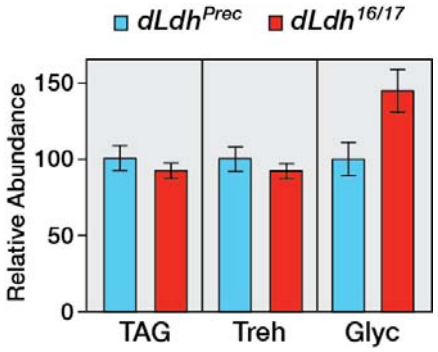


Macromolecule biosynthesis is largely normal in *Ldh* mutants



Ldh mutants exhibit a modest increase in glycogen.

The *Ldh* mutant phenotype is reminiscent of Glycogen Storage Disease Type XI

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GTR: GENETIC TESTING REGISTRY

Conditions/Phenotypes Search

GTR Home > **Conditions/Phenotypes** > Glycogen storage disease XI

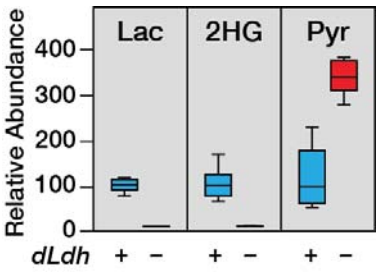
Glycogen storage disease XI

Synonyms: GSD XI; LACTATE DEHYDROGENASE A DEFICIENCY
Modes of inheritance: Autosomal recessive inheritance (HPO, OMIM, Orphanet)

Summary

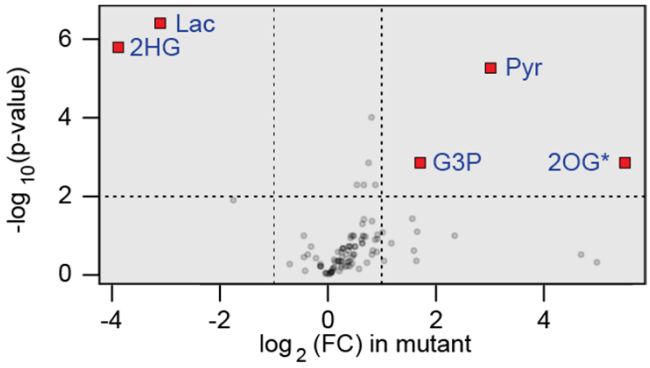
Lactate dehydrogenase deficiency is a condition that affects how the body breaks down sugar to use as energy in cells, primarily muscle cells.There are two types of this condition: lactate dehydrogenase-A deficiency (sometimes called glycogen storage disease XI) and lactate dehydrogenase-B deficiency.People with lactate dehydrogenase-A deficiency experience fatigue, muscle pain, and cramps during exercise (exercise intolerance). In some people with lactate dehydrogenase-A deficiency, high-intensity exercise or other strenuous activity leads to the breakdown of muscle tissue (rhabdomyolysis). The destruction of muscle tissue releases a protein called myoglobin, which is processed by the kidneys and released in the urine (myoglobinuria). Myoglobin causes the urine to be red or brown. This protein can also damage the kidneys, in some cases leading to life-threatening kidney failure. Some people with lactate dehydrogenase-A deficiency develop skin rashes. The severity of the signs and symptoms among individuals with lactate dehydrogenase-A deficiency varies greatly.People with lactate dehydrogenase-B deficiency typically do not have any signs or symptoms of the condition. They do not have difficulty with physical activity or any specific physical features related to the condition. Affected individuals are usually discovered only when routine blood tests reveal reduced lactate dehydrogenase activity. [from GTR]

Metabolomic analysis of *Ldh* mutants



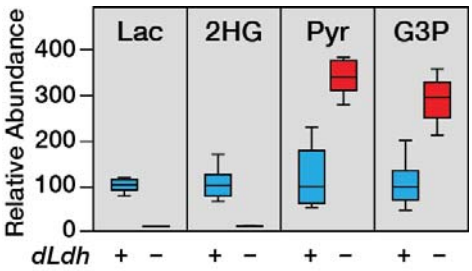
Ldh mutants display the expected changes in lactate, pyruvate, and 2HG.

Metabolomic analysis of *Ldh* mutants



Relatively few metabolites changed in response to loss of LDH activity

Ldh mutants exhibit increased Glycerol-3-phosphate production



Ldh mutants also display increased G3P.

Ldh mutants exhibit increased GPDH expression

Table 2. Metabolic Genes that are significantly misregulated in *Ldh* mutants

Gene Name	Function	Fold Change
Dot	UDP-glucuronosyl/UDP-glucosyltransferase	3.7
Gpdh	**Glycerol-3-phosphate dehydrogenase**	**3.6**
Ucp4B	Uncoupling protein 4B	3.3
CG34345	Oxoglutarate/iron-dependent dioxygenase	3.2
Orct	Solute Carrier Family	2.7
CG11208	2-hydroxyacyl-CoA lyase	2.7
Cyp6d5	Cytochrome P450	2.5
CG8008	Solute Carrier Family	2.4
MFS16	Solute Carrier Family 37A2	2.3
CG13248	Solute Carrier Family 7A4	2.3
PH4alphaMP	prolyl-4-hydroxylase-alpha MP	2.1
Cyp12a5	Cytochrome P450	-2.2
CG2065	short chain dehydrogenase	-2.5
Gpi1	N-acetylglucosaminyl transferase	-2.5
ImpL3	Lactate Dehydrogenase	-3.7
Sodh-2	Sorbitol dehydrogenase-2	-4.9

LDHA inhibition in pancreatic cancer cells also results in elevated G3P synthesis

nature chemical biology

ARTICLE

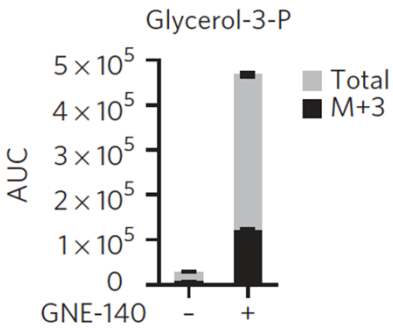
PUBLISHED ONLINE: 1 AUGUST 2016 | DOI: 10.1038/NCHEMBIO.2143

Metabolic plasticity underpins innate and acquired resistance to LDHA inhibition

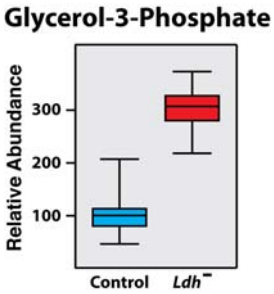
Aaron Boudreau[1,13], Hans E Purkey[2,13], Anna Hitz[3], Kirk Robarge[2], David Peterson[1], Sharada Labadie[2], Mandy Kwong[3], Rebecca Hong[3], Min Gao[3], Christopher Del Nagro[3], Raju Pusapati[1], Shuguang Ma[4], Laurent Salphati[4], Jodie Pang[4], Aihe Zhou[2], Tommy Lai[5], Yingjie Li[6], Zhongguo Chen[6], Binqing Wei[2], Ivana Yen[7], Steve Sideris[7], Mark McCleland[8], Ron Firestein[8], Laura Corson[3], Alex Vanderbilt[9], Simon Williams[9], Anneleen Daemen[10], Marcia Belvin[3], Charles Eigenbrot[11], Peter K Jackson[3,12], Shiva Malek[7], Georgia Hatzivassiliou[3], Deepak Sampath[3], Marie Evangelista[1]* & Thomas O'Brien[3]*

LDH and G3P link in tumors *in vitro* and *in vivo*

In vitro **LDHA** inhibition:

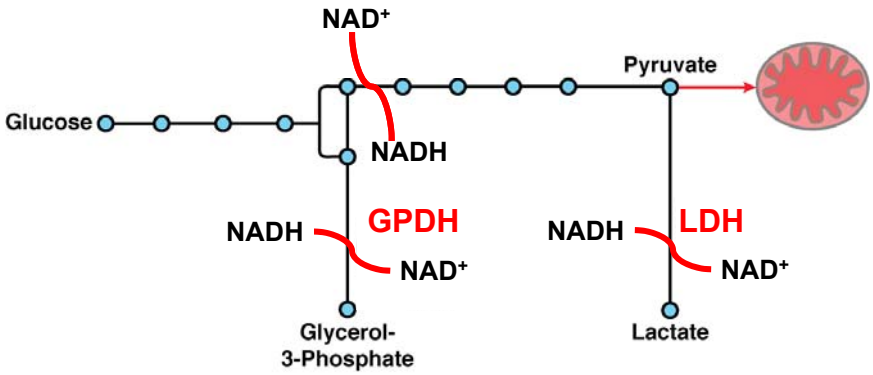


In vivo **LDH** inhibition:

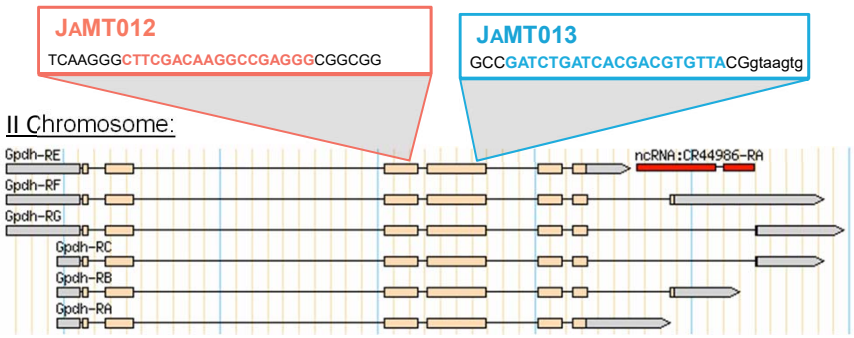


Boudreau A, Metabolic plasticity underpins innate and acquired resistance to LDHA inhibition. Nat Chem Biol. 2016 Aug 1. doi: 10.1038/nchembio.2143

Glycerol 3-phosphate production could compensate for loss of LDH

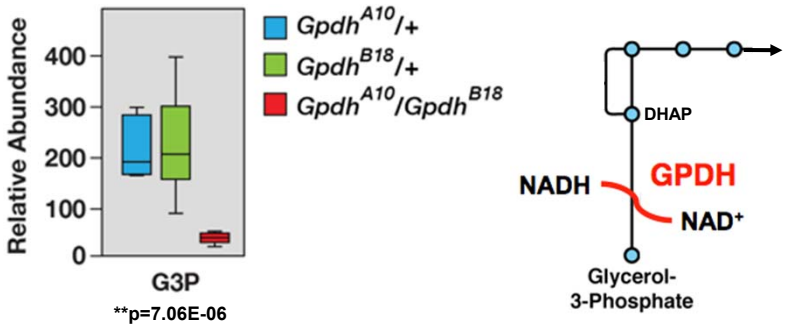


GPDH CRISPR deletions successfully made in the III and IV exon

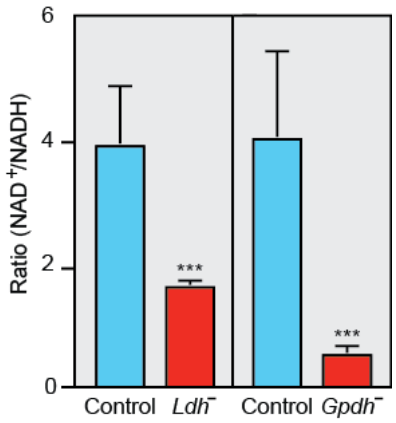


CRISPR A10	**deletion of 19**	JaMT012	TCAAGGGCTTCGACAAGGCCGAGGGCGGCGG
CRISPR B26	**deletion of 2**	JaMT012	TCAAGGGCTTCGACAAGGCCGAGGGCGGCGG
CRISPR B18	**deletion of 7**	JaMT013	GCCGATCTGATCACGACGTGTTACGgtaagtg

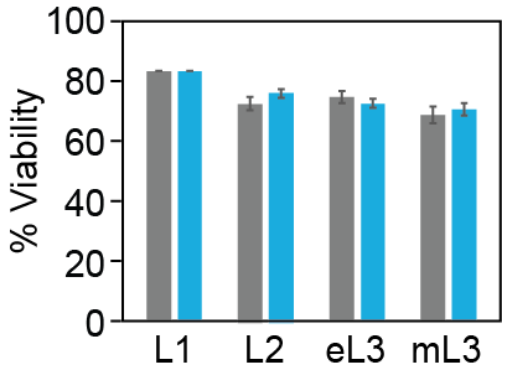
GPDH CRISPR mutants exhibit significant decreases in glycerol-3-phosphate accumulation



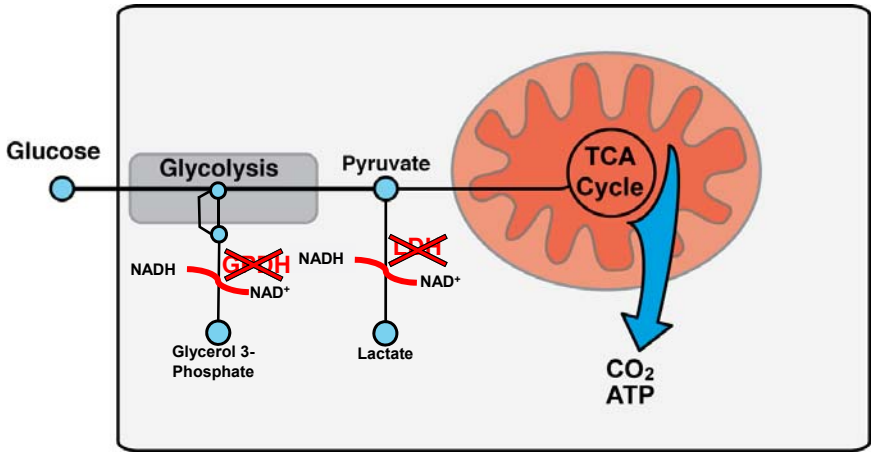
GPDH is required to maintain larval redox balance



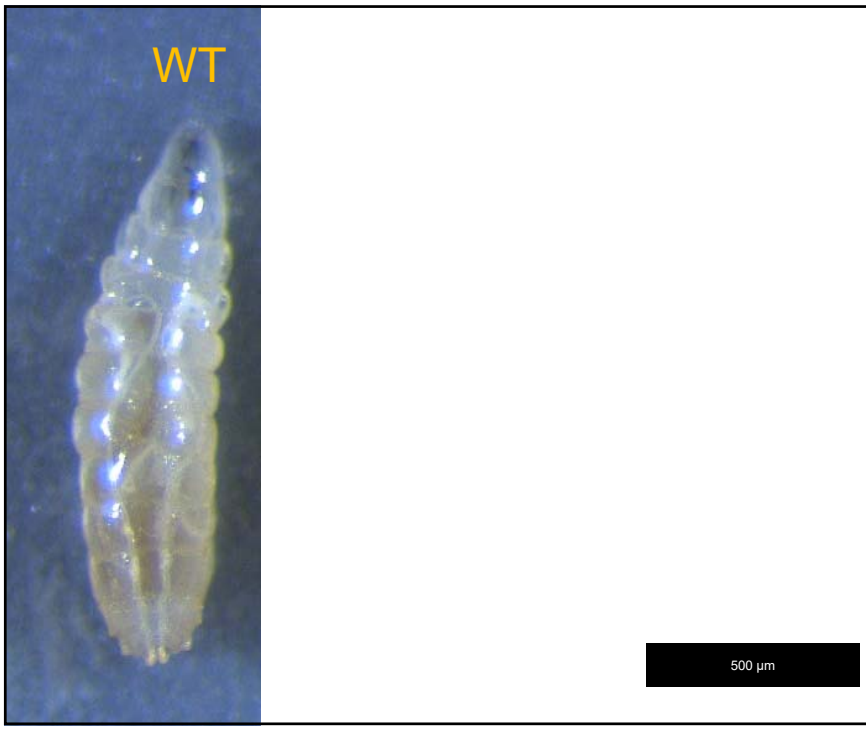
***Gpdh* mutants grow at a normal rate**

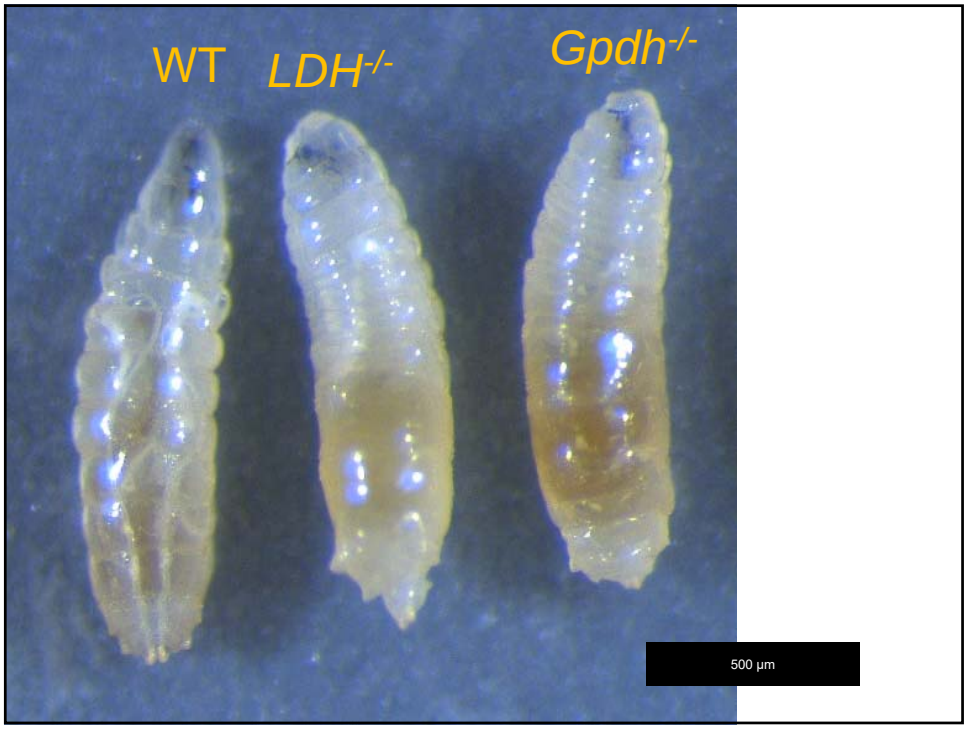


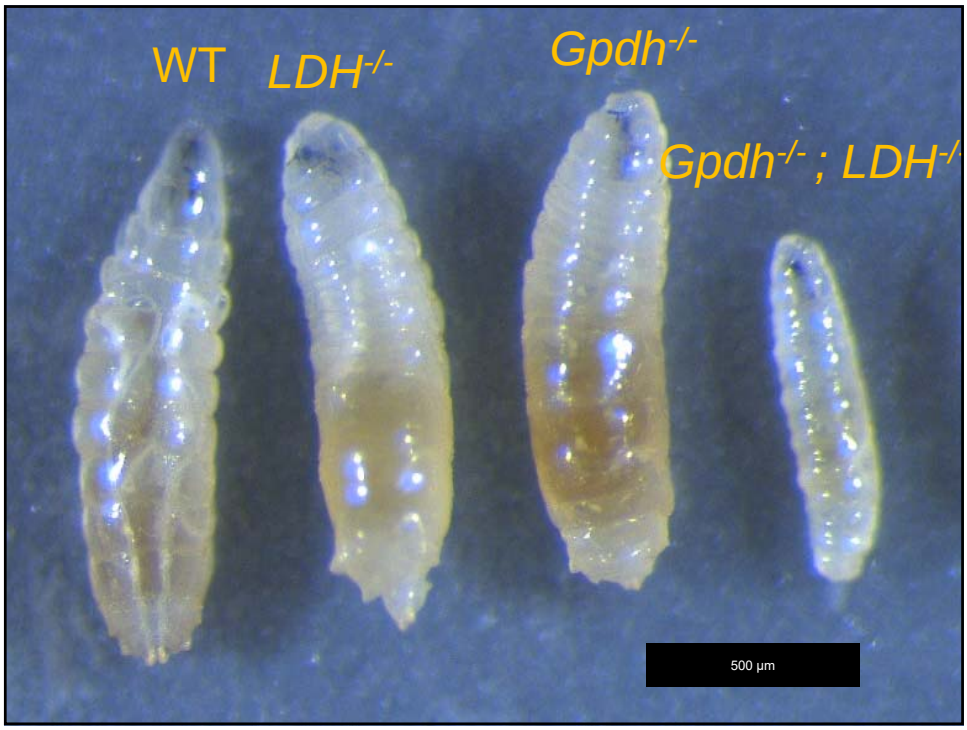
Functional significance of GPDH when LDH is mutated



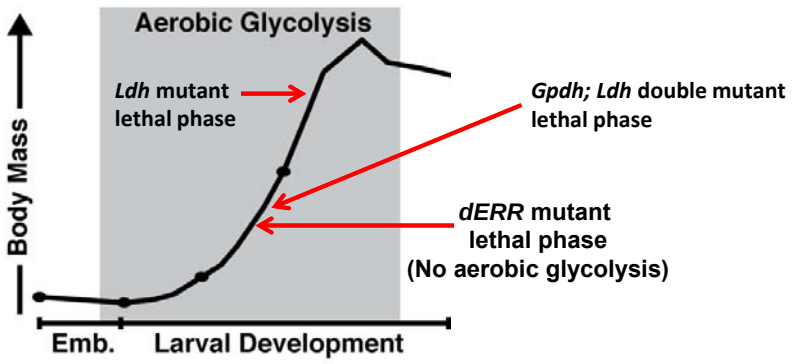
**Expect lethality if GPDH is compensating for LDH loss by regenerating NAD+*




WT
LDH-/-
Gpdh-/-
500 µm


WT
LDH-/-
Gpdh-/-
Gpdh-/- ; LDH-/-
500 µm

Gpdh; Ldh double mutants exhibit a synthetic lethal phenotype



The relationship between LDH and GPDH was observed in tumors nearly 60 years ago

Low Levels of Soluble DPN-linked α-Glycerophosphate Dehydrogenase in Tumors*

GEORGE E. BOXER AND CARL E. SHONK

(*Merck Institute for Therapeutic Research, Rahway, N.J.*)

GPDH AND LDH ACTIVITY OF NORMAL TISSUES

The ranges indicated refer to the highest and lowest values observed. The single values are averages of three or less determinations.

Species	Tissue	GPDH (units/mg)	LDH (units/mg)	LDH/GPDH
Rat	Liver	90–225	238–322	1.3–2.6
	Muscle	176–639	472–1528	2.7–3.4
	Lymph nodes	75–150	100–200	0.5–4.0
	Adipose tissue	50	65	1.3
	Brain	31–42	128–132	3.1–4.3
Mouse	Liver	112–181	294–500	1.6–3.4
	Kidney	158	132	0.8
	Spleen	28	22	0.8
	Muscle	188–263	1350–1514	5.1–7.2
	Brain	31–53	161–172	3.0–5.3
Hamster	Liver	192–245	126–167	0.55–0.68
	Liver*	143–197	79–85	0.43–0.55
	Kidney	138	93	0.67
	Kidney*	100–119	62–81	0.62–0.68
	Spleen	64	34	0.53
	Muscle	158–274	964–1585	5.0–7.0

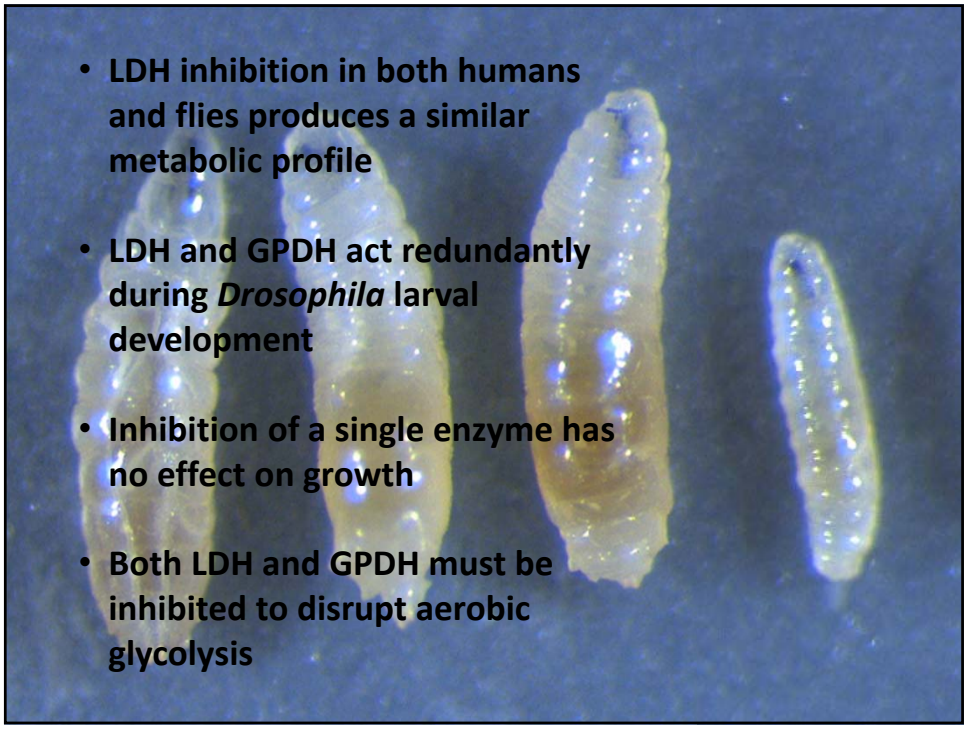
* Data for hamsters under cortisone treatment.

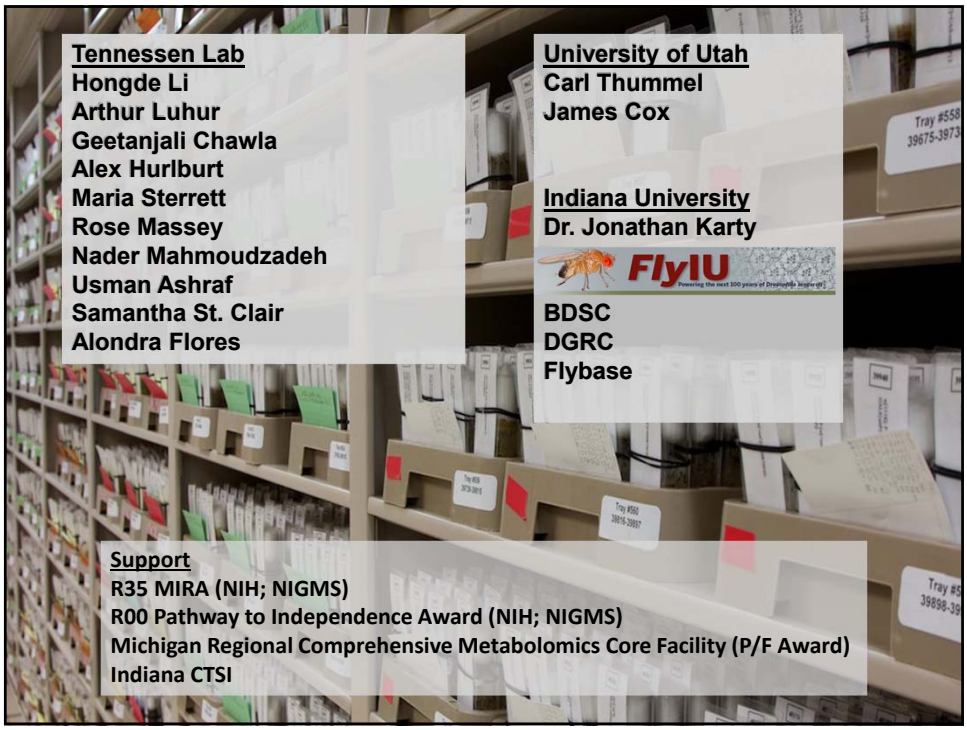
GPDH AND LDH ACTIVITY IN TRANSPLANTED RAT AND HAMSTER TUMORS AND OF HUMAN TUMORS GROWING IN CORTISONIZED HAMSTERS

Tumors	GPDH (units/mg)	LDH (units/mg)	LDH/GPDH
Rat:			
Murphy lymphosarcoma	8	94	12
Walker 256	8	278	35
Jensen sarcoma	3	123	41
Hamster:			
Fortner, pancreatic #1*	3	470	157
Fortner, small bowel carcinoma*	5	422	84
Bashford carcinoma*	14	332	24
Crabb sarcoma	> 0.7	660	<900
Human (I.M. in cortisonized hamsters):			
Adenocarcinoma #1	7	298	43
Epidermoid carcinoma #3	4	145	36
Bronchiogenic carcinoma	25	234	9
Sarcoma #1	5	210	42

The values represent averages of results obtained on two or more individual tumors, except those indicated by an asterisk, which were obtained on single tumors.

Cancer Research (1960) 20:85-91


• LDH inhibition in both humans and flies produces a similar metabolic profile
• LDH and GPDH act redundantly during *Drosophila* larval development
• Inhibition of a single enzyme has no effect on growth
• Both LDH and GPDH must be inhibited to disrupt aerobic glycolysis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