Studies of Cancer Metabolism using *Drosophila* as a Model System

Jason M. Tennesen
Indiana University

Tumor cells use aerobic glycolysis to generate energy

![Diagram showing glucose metabolism in quiescent and tumor cells]
Tumor cells use aerobic glycolysis to generate energy and biomass

Glycolysis fuels oxidative metabolism
Aerobic glycolysis supports biosynthesis

Glucose → Glycolysis → Pyruvate → TCA Cycle → CO₂, ATP

Aerobic glycolysis supports biosynthesis

Glucose → Glycolysis → Pyruvate → TCA Cycle → CO₂, ATP

Pentose Phosphate Pathway
DNA, RNA
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**

*Drosophila* Life Cycle

Body Mass

Emb.  Larval Development  Metamorphosis  Adult
**dERR mutants are energy starved and hyperglycemic**

![Graph showing ATP and circulating sugar levels compared to control](image)

*Genomic and metabolomic analysis of dERR mutants*
**dERR mutants do not use sugar to generate energy**

Glucose → Glycolysis → Pyruvate → TCA Cycle → CO₂, ATP

**dERR mutants display reduced expression of genes in the glycolytic pathway**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>HexA</td>
<td>-1.7</td>
</tr>
<tr>
<td>Pgi</td>
<td>-5.2</td>
</tr>
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**Predicted enzyme**

<table>
<thead>
<tr>
<th>Predicted enzyme</th>
<th>Gene</th>
<th>Fold-Change</th>
<th>ERR binding site(s)</th>
</tr>
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<tbody>
<tr>
<td>Hexokinase</td>
<td>CG3001 (HexA)</td>
<td>-1.7</td>
<td>-913 aAAGGTCA -128 TAAGGTCA</td>
</tr>
<tr>
<td>Phosphoglucone isomerase</td>
<td>CG8251 (Pgi)</td>
<td>-5.2</td>
<td>+871 ggAAGGTCA +998 cgAGGTCA +1500 TAAGGTCA</td>
</tr>
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<td>Aldolase</td>
<td>CG6258 (Ald)</td>
<td>-3.5</td>
<td>-636 aAgAAGGTCA +541 caAAGGTCA +852 cCAaGTCA</td>
</tr>
<tr>
<td>Triosephosphate isomerase</td>
<td>CG2771 (Tpi)</td>
<td>-3.1</td>
<td>-678 cAAGGTCA</td>
</tr>
<tr>
<td>Glyceroldehyde phosphate dehydrogenase</td>
<td>CG8893 (Gapdh2)</td>
<td>-3.1</td>
<td>-847 aaAAGGTCA -489 TgaAGGTCA -192 TsAAGGTCA</td>
</tr>
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<td>Phosphoglycerate kinase</td>
<td>CG3727 (Pgk)</td>
<td>-3.7</td>
<td>+16 gcAAGGTCA</td>
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<tr>
<td>Phosphoglycerate mutase</td>
<td>CG1721 (Pglym78)</td>
<td>-3.8</td>
<td>-209 aaAAGGTCA +103 TgaAGGTCA +446 aCAaGTCA</td>
</tr>
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<td>Enolase</td>
<td>CG17654 (Eno)</td>
<td>-1.9</td>
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<td>Pyruvate kinase</td>
<td>CG7970 (Pyk)</td>
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**dERR directly regulates genes in the glycolytic pathway**

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dERR binds to a canonical site in *Pfk*

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*Phosphofructokinase*

**dERR ChIP**

**Mock IP**

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**dERR binds to a canonical site in *Pfk***

**Phosphofructokinase**

TGGCCTGAAGGTCACCTTG
The dERR binding site in Pfk is conserved across species

Phosphofructokinase

D. melanogaster  TGGCCTGAAGGTCACCTTG
D. simulans  TGGCCCTGAAGGTCACCTTG
D. sechellia  TGGCCCTGAAGGTCACCTTG
D. erecta  TGGCCCTGAAGGTCACCTTG
D. yakuba  TGGCCTGAAGGTCACCTTG
D. ananassae  TCCCCAAAAGGTCACCTTG
D. virilis  GCGTTTAAAGGTCACCTTG
D. mojavensis  GCGTTTAAAGGTCACCTTG
D. grimshawi  GCGTTTAAAGGTCACCTTG

dERR mutants display reduced expression of genes in the glycolytic pathway

Glycolysis
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Pgi -5.2
Pfk -6.9
Ald -3.5
Tpi -3.1
Gapdh2 -3.1
Pgk -3.7
Pgym78 -3.8
Eno -1.9
Pyk -1.7
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
Proline is depleted in \textit{dERR} mutants

\textit{dERR} mutant phenotypes suggest that \textit{Drosophila} larvae use aerobic glycolysis
$dERR$ mutant phenotypes suggest that *Drosophila* larvae use aerobic glycolysis.

* Drosophila larvae 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.

control

dERR−
Conclusions

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

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

Cancer cells typically display altered glucose metabolism characterized by a preference of anaerobic glycolysis, known as the Warburg effect, which facilitates cell proliferation. Hypoxia-inducible factor (HIF) and coactivator p300 are two prominent transcription factors that drive glycolysis. Previously, we reported that the estrogen-related receptor ERRalpha acts as a cofactor of HIF and enhances HIF-dependent transcription of glycolytic genes under hypoxic conditions. 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 ERRalpha stimulates glycolysis under normoxic conditions. 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 HIF and the two factors synergistically activate transcription of glycolytic genes. Furthermore, overexpression of ERRalpha increases glycolytic gene expression and lactate production. Conversely, depletion of ERRs in cancer cells represses 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.

Keywords: aerobic glycolysis; Warburg effect; nuclear receptor; mitochondrial biogenesis.
What is the role of LDH in promoting larval growth?

Ldh mutants fail to produce lactate

<table>
<thead>
<tr>
<th>Relative Abundance</th>
<th>Control</th>
<th>Ldh^-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

Glucose → NADH → Pyruvate → LDH → NAD+ → Lactate

Amino Acid Synthesis

Pentose Phosphate Pathway
Pyruvate levels are elevated in $Ldh$ mutants

$Ldh$ mutants fail to regenerate NAD$^+$
*Ldh* mutants die near the end of larval growth

*Ldh* mutant lethal phase

*Ldh* mutants grow at a normal rate

*Aerobic Glycolysis*
**Ldh mutants die near the end of larval growth**

![Graph showing body mass over larval development with Ldh mutant and dERR mutant lethal phases.](image)

- Ldh mutant lethal phase
- dERR mutant lethal phase (No aerobic glycolysis)

**Macromolecule biosynthesis is largely normal in Ldh mutants**

![Bar chart showing relative abundance of TAG, Treh, and Glyc in dLdh\textsuperscript{Proc} and dLdh\textsuperscript{10/17}.](image)

- Ldh mutants exhibit a modest increase in glycogen.
The *Ldh* mutant phenotype is reminiscent of Glycogen Storage Disease Type XI

**Glycogen storage disease XI**

**Synonyms:**
- GSD XI, LACTATE DEHYDROGENASE A DEFICIENCY

**Modes of inheritance:**
- Autosomal recessive inheritance (AR, 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 can lead 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: 6)

**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

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Function</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dot</td>
<td>UDP-glucuronosyl/UDP-glucosyltransferase</td>
<td>3.7</td>
</tr>
<tr>
<td>Gpdh</td>
<td>Glycerol-3-phosphate dehydrogenase</td>
<td>3.6</td>
</tr>
<tr>
<td>Ucp4B</td>
<td>Uncoupling protein 4B</td>
<td>3.3</td>
</tr>
<tr>
<td>CG34345</td>
<td>Oxoglutarate/iron-dependent dioxygenase</td>
<td>3.2</td>
</tr>
<tr>
<td>Orct</td>
<td>Solute Carrier Family</td>
<td>2.7</td>
</tr>
<tr>
<td>CG11208</td>
<td>2-hydroxacyl-CoA lyase</td>
<td>2.7</td>
</tr>
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<td>Cyp6d5</td>
<td>Cytochrome P450</td>
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<td>CG8008</td>
<td>Solute Carrier Family</td>
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<td>MFS16</td>
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<tr>
<td>CG13248</td>
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<td>PH4alphaMP</td>
<td>prolyl-4-hydroxylase-alpha MP</td>
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<tr>
<td>Cyp12a5</td>
<td>Cytochrome P450</td>
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<tr>
<td>CG2065</td>
<td>short chain dehydrogenase</td>
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<tr>
<td>Gpi1</td>
<td>N-acetylglucosaminyl transferase</td>
<td>-2.5</td>
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<tr>
<td>ImpL3</td>
<td>Lactate Dehydrogenase</td>
<td>-3.7</td>
</tr>
<tr>
<td>Sodh-2</td>
<td>Sorbitol dehydrogenase-2</td>
<td>-4.9</td>
</tr>
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</table>

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

Metabolic plasticity underpins innate and acquired resistance to LDHA inhibition

Aaron Boudreau, Hans E Purkey, Anna Hilt, Kirk Robarge, David Peterson, Sharada Labadie, Mandy Kwong, Rebecca Hong, Min Gao, Christopher Del Nagro, Raju Pusapati, Shuqing Ma, Laurent Salphati, Jodie Pang, Aihe Zhou, Tommy La, Yingjie Li, Zhongguo Chen, Bingjing Wu, Ivana Yan, Steve Sideris, Mark McCleland, Ron Firestein, Laura Corson, Alex Vanderbilt, Simon Williams, Anneliese Daemen, Marcia Belvin, Charles Eisenbrodt, Peter K Jackson, Shiva Malek, Georgia Hatzivassiliou, Deepak Sampath, Marie Evangelista & Thomas O'Brien
LDH and G3P link in tumors *in vitro* and *in vivo*

*In vitro* LDHA inhibition:

- Glycerol-3-P (AUC)
  - 5 x 10^5
  - 4 x 10^5
  - 3 x 10^5
  - 2 x 10^5
  - 1 x 10^5
  - 0
- GNE-140:
  - -
  - +

*In vivo* LDH inhibition:

Glycerol-3-Phosphate

- Relative Abundance
  - Control
  - Ldh^-^-

Glycerol 3-phosphate production could compensate for loss of LDH

GPDH CRISPR deletions successfully made in the III and IV exon

II Chromosome:

CRISPR A10 deletion of 19 JAMT012
TCAAGGGCTTCGACAAGGCCGAGGGCGG

CRISPR B26 deletion of 2 JAMT012
TCAAGGGCTTCGACAAGGCCGAGGGCGG

CRISPR B18 deletion of 7 JAMT013
GCCGATCTGATCACGACGTGTTAGtgaagtg
GCCGATCTGATCACGACGTGTTA

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

**p=7.06E-06
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+*
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

(Mercer Institute for Therapeutic Research, Princeton, N.J.)

<table>
<thead>
<tr>
<th>Species</th>
<th>Tumor</th>
<th>GPDH (units/mg)</th>
<th>LDH (units/mg)</th>
<th>LDH/GPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Liver</td>
<td>98-1529</td>
<td>478-1280</td>
<td>0.5-2.3</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>156-250</td>
<td>451-1200</td>
<td>0.5-2.3</td>
</tr>
<tr>
<td></td>
<td>Adipose tissue</td>
<td>80-160</td>
<td>240-520</td>
<td>2.3-4.5</td>
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<tr>
<td></td>
<td>Brain</td>
<td>32-63</td>
<td>140-250</td>
<td>0.3-0.5</td>
</tr>
<tr>
<td>Mouse</td>
<td>Liver</td>
<td>110-161</td>
<td>686-900</td>
<td>1.6-6.3</td>
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<tr>
<td></td>
<td>Kidney</td>
<td>120</td>
<td>102</td>
<td>1.5-4.3</td>
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<tr>
<td></td>
<td>Spleen</td>
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<td>1.7-3.3</td>
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<td>0.2-0.3</td>
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<tr>
<td>Hamster</td>
<td>Liver</td>
<td>150-254</td>
<td>180-170</td>
<td>0.3-4.6-7.9</td>
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<tr>
<td></td>
<td>Kidney*</td>
<td>120-198</td>
<td>90-160</td>
<td>0.42-6.32</td>
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<td>60-120</td>
<td>160-170</td>
<td>0.08-0.81</td>
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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.

* Data for hamsters under cortisone treatment.

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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Support

R35 MIRA (NIH; NIGMS)
R00 Pathway to Independence Award (NIH; NIGMS)
Michigan Regional Comprehensive Metabolomics Core Facility (P/F Award)
Indiana CTSI

Tennessen Lab
Hongde Li
Arthur Luhur
Geetanjali Chawla
Alex Hurlburt
Maria Sterrett
Rose Massey
Nader Mahmoudzadeh
Usman Ashraf
Samantha St. Clair
Alondra Flores

University of Utah
Carl Thummel
James Cox

Indiana University
Dr. Jonathan Karty

BDSC
DGRC
Flybase

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