Effect of PDK activation and PDH flux in Metabolic Syndrome;

New Therapeutic Target of Metabolic syndrome and Cancer

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Obesity is linked to the development of many chronic diseases

- Adipocytes
- Immune cells
- Brain cells
- Systemic and local increase in cytokine concentrations
- Glucose
  - Insulin resistance
  - Type 2 diabetes
- Atherosclerosis
- Alzheimer’s disease
- Huntington’s disease
- Parkinson’s disease
- Cancer

metaflammation
“Healthy” cells, which mainly generate energy from oxidative breakdown of pyruvate in mitochondria. “Aerobic glycolysis” (generation of lactate in the presence of oxygen) is important for cancer cells.
Background of Pyruvate Dehydrogenase Complex
Mitochondria and PDH

• PDH: Large complex containing many copies of 3 enzymes, $E_1$, $E_2$, & $E_3$.
• The inner core of PDH is an icosahedral st. consist of 60 copies of $E_2$.

- At the periphery of the complex are:
  - 30 copies of $E_1$ (itself a tetramer with subunits $a_2b_2$).
  - 12 copies of $E_3$ (a homodimer), plus 12 copies of an $E_3$ binding protein that links $E_3$ to $E_2$. 
PDK activation involves interaction with $E_2$ subunits to sense changes in oxidation state & acetylation of lipoamide caused by NADH & acetyl-CoA.
Regulation of pyruvate dehydrogenase complex (PDC) activity by its kinases and phosphatase

- Insulin (-)
- Starvation/Diabetes (+)
- PDK1 (-)
- PDK2 (+)
- PDK3 (-)
- PDK4 (+)
- ADP
- ATP
- H₂O
- Pi
- CO₂
- Acetyl-CoA
- NAD⁺
- NADH
- CoASH
- Pyruvate
- PDC (Inactive) → OP
- PDC (Active)
- OH
PDK in Cancer Cells
PDK in Metabolic Syndrome (Obesity, DM)
PDK in Vascular Calcification
In cancer cells and tumors, high lactate generation and low glucose oxidation, despite normal oxygen conditions, are commonly seen. Historically known as the Warburg effect, this altered metabolic phenotype has long been correlated with malignant progression and poor clinical outcome.
PDK in cancer cells

Effect of PDK1 knockdown on cancer growth

PDK in cancer cells

- PDK4 is overexpressed in a subset of human cancers and contributes to anoikis resistance in cancer cells
- Anoikis (Matrix detachment-induced apoptosis)

Depletion of PDK4 or activation of PDH increased mitochondrial respiration and oxidative stress in suspended cells, resulting in heightened anoikis. Conversely, overexpression of PDKs prolonged survival of cells in suspension.
PDK in Cancer Cells
PDK in Metabolic Syndrome (Obesity, DM)
PDK in Vascular Calcification
(Question 1)

What is the Role of PDK4/2 on blood Glucose?
Effect of Starvation and Diabetes on PDK2 and PDK4 Protein in Rat skeletal muscle

Effect of Starvation on PDK2 and PDK4 Protein in Liver of wild and PDK4

**Wild type** | **PDK4**
---|---
PDHK4 | Fed | Starved | Fed | Starved | PDH E1α
PDHK2
PDH E1α

Pyruvate Dehydrogenase Complex (PDC)

- Fed state; PDC is active for complete oxidation of glucose and fat synthesis
- Fasting state; PDC is inhibited, lactate, alanine and pyruvate used for gluconeogenesis

Robert A. Harris
(Question 2)

Does knocking out PDK4 decrease blood glucose level? And how?
## Blood Levels of Glucose in WT and KO Mice

<table>
<thead>
<tr>
<th>Metabolic State</th>
<th>Blood from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDK4&lt;sup&gt;+/+&lt;/sup&gt; mice</td>
</tr>
<tr>
<td>Fed (mg/dL)</td>
<td>140 ± 8</td>
</tr>
<tr>
<td>Fasted overnight (mg/dL)</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>Starved 48 hours (mg/dL)</td>
<td>139 ± 5</td>
</tr>
</tbody>
</table>

*P<0.01

Blood Levels of Three Carbon Compounds in WT and KO Mice

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Blood from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDK4&lt;sup&gt;+/+ &lt;/sup&gt;mice</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>1.93 ± 0.15</td>
</tr>
<tr>
<td>Pyruvate (mmol/l)</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Alanine (mmol/l)</td>
<td>0.26 ± 0.01</td>
</tr>
</tbody>
</table>

*P<0.01

Effect of starvation on levels of lipids metabolites in wild-type and PDK4 KO mice

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Blood from:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild-type</td>
<td>PDK4^-/-</td>
</tr>
<tr>
<td>Free fatty acids (mmol/l)</td>
<td>1.44 ± 0.13</td>
<td>2.20 ± 0.07*</td>
</tr>
<tr>
<td>3-Hydroxybutyrate (mmol/l)</td>
<td>1.90 ± 0.60</td>
<td>6.38 ± 1.81*</td>
</tr>
<tr>
<td>Acetoacetate (mmol/l)</td>
<td>0.14 ± 0.04</td>
<td>1.28 ± 0.11*</td>
</tr>
</tbody>
</table>

Q2) How does knocking out PDK4 decrease blood glucose level?

- **Answer:**
  - Greater PDC activity promotes oxidation of pyruvate
  - Increased rate of pyruvate oxidation inhibits generation of pyruvate (and lactate and alanine) by glycolysis
(Question 3)

What is the Role of PDK4 in high fat feeding diet?

Fed wild type and PDK4 knockout mice for 18 weeks on high fat (58% calorie basis) diet
Glucose Tolerance Test on High Fat Fed Mice (n=5 mice/group)

Lower body and adipose tissue weights in PDK4\(^{-/-}\) mice fed high fat diet

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Body weight</th>
<th>Epididymal fat pads</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDK4(^{+/+})</td>
<td>48.3 ± 1.8</td>
<td>3.59 ± 0.11</td>
</tr>
<tr>
<td>PDK4(^{-/-})</td>
<td>41.1 ± 1.5(^*)</td>
<td>2.68 ± 0.12(^*)</td>
</tr>
</tbody>
</table>

N= 6 mice each group; *P < 0.05

Summary(1)

- Knocking out PDK4 results in lower fasting blood glucose levels and modestly improved glucose tolerance and insulin sensitivity.

- Knocking out PDK4 results in lower body weight and less body fat in high fat fed mice.

Robert A. Harris
Effect of DIO and Diabetes on PDK2 and PDK4 Expression in Liver

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(Question 4)

What about PDK2?
What about PDK2?

- PDK2 constitutively expressed in most tissues.
- PDK2 expression increased in liver and kidney in response to fasting and diabetes.
- We therefore generated PDK2 knockout mouse to determine the effect on glucose homeostasis.
Effect of the high fat diet on growth curve of wild-type and PDK2\(^{-/-}\) mice

16 week Model

7-13 mice per each group

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### Fasting blood metabolite levels of PDK2<sup>−/−</sup> mice in diet induced obese (DIO) mice

<table>
<thead>
<tr>
<th>Measurement†</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild-type</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>179.8 ± 8.8</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.12 ± 0.10</td>
</tr>
<tr>
<td>Pyruvate (mmol/L)</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>3-Hydroxybutyrate (mmol/L)</td>
<td>1.04 ± 0.12</td>
</tr>
<tr>
<td>Acetoacetate (mmol/L)</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Free fatty acids (mmol/L)</td>
<td>0.21 ± 0.02</td>
</tr>
</tbody>
</table>

*Significant difference from Wild-type

†Measurement at fasting state

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Glucose Tolerance Test of PDK2\(^{-/-}\) mice in DIO

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Metabolic profiles of wild-type and PDK2\(^{-/-}\) mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control diet</th>
<th>High fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wild-type</td>
<td>PDK2(^{-/-})</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>94.5±0.5</td>
<td>92.8±2.9</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>1.3±0.1</td>
<td>0.9±0.4</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>5.4±0.1</td>
<td>5.0±0.1</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>119.0±2.7</td>
<td>110.0±3.9</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>64.0±1.0</td>
<td>54.5±3.2 (^#)</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>4.4±0.4</td>
<td>5.9±0.5</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>51.4±3.1</td>
<td>34.3±1.1 (^{**})</td>
</tr>
<tr>
<td>Free fatty acid (mM)</td>
<td>0.12±0.02</td>
<td>0.29±0.03 (^#)</td>
</tr>
<tr>
<td>Adiponectin (mg/dL)</td>
<td>11.7±1.1</td>
<td>11.9±0.8</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>2.0±0.2</td>
<td>2.7±1.9</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>4.8±0.6</td>
<td>3.5±0.4</td>
</tr>
<tr>
<td>Pyruvate (mM)</td>
<td>0.17±0.02</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>1.32±0.02</td>
<td>1.25±0.02 (^#)</td>
</tr>
</tbody>
</table>

16 week Model

WT-CD vs PDK2\(^{-/-}\)-CD; \(^#\)P<0.05, \(^{**}\)P<0.01
WT-HFD vs PDK2\(^{-/-}\)-HFD; \(^*\)P<0.05, \(^{**}\)P<0.01, \(^{***}\)P<0.001

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Effect of PDK2 deficiency on hepatic steatosis induced by the high fat diet

Lab of endocrinology
Effect of PDK2 deficiency on hepatic steatosis induced by the high fat diet

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PDK2 deficiency suppresses fat synthesis

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PDK2 deficiency activates FA oxidation and ketogenesis

Liver

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Improvement of hepatic glucose homeostasis in PDK2 deficiency mice

Basal

Clamp

Hepatic glucose production (mg/kg/min)

Relative mRNA levels

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Reductions of the anaplerotic influx and the TCA cycle intermediates in PDK2 deficiency mice

**Serum**
- Pyruvate (mM)

**Liver**
- Oxaloacetate
- Citrate
- Succinate

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PDK2 Knock-Down Increased Beta-oxidation by XF-analyzer
HFD-induced hepatic insulin resistance is ameliorated by PDK2 deficiency due to the decreased diacylglycerol (DAG)
Hepatic insulin signaling pathway in liver of HFD fed WT and PDK2-/- overnight-fasted mice after IP injection of saline and insulin (5U/kg body weight).
Knocking out PDK2 in the Liver

Gluconeogenesis

Glucose

Ketones

G-6-Pase

PEP

PK

PEPCK

Lactate

Pyruvate

Alanine

Oxaloacetate

MDH 2

MDH 1

Malate

Malate

Mitochondria

Oxaloacetate

Pyruvate

Pyruvate Dehydrogenase

Acetyl-CoA

Ketogenesis

B-Oxidation

CPT-1

Free Fatty Acids

Glycolysis occurs in the cytosol. Pyruvate enters mitochondrion, Pyruvate Dehydrogenase, catalyzes oxidative decarboxylation of pyruvate, to form acetyl-CoA.
• Knocking out PDK2 results in lower fasting blood glucose levels and improved glucose tolerance and insulin sensitivity.

• Knocking out PDK2 results in lower body weight and less body fat in high fat fed mice.

• Knocking out PDK2 results in lower cholesterol, greater adiponectin level, and markedly improved fatty infiltration of liver.
Question 5)

Are the PDKs viable targets for the treatment of Type 2 Diabetes?
Are the PDKs viable targets for the treatment of Type 2 Diabetes?

• **On the positive side**
  Compounds that inhibit PDKS should lower blood glucose, improve glucose tolerance, and decreased body fat.

• **On the negative side**
  Ketone bodies will be increased.
# PDK4 Inhibitor

<table>
<thead>
<tr>
<th>상태</th>
<th>물질</th>
<th>회사</th>
<th>작용기전</th>
<th>작용질환</th>
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<tbody>
<tr>
<td>Phase III</td>
<td>CPC-2011 DCA X-115</td>
<td>Questcor University of Alberta University of California, San Diego University of Cincinnati</td>
<td>Pyruvate Dehydrogenase Kinase (PDK; PDK) Inhibitors</td>
<td>Ischemic Stroke, Neurologic disease, Solid Tumors and Brain Tumors, Brain cancer Therapy, Disorders of the Coronary Arteries and Atherosclerosis</td>
</tr>
<tr>
<td>Preclinical</td>
<td>NSC-294404</td>
<td>Kitasato Institute</td>
<td>Pyruvate Dehydrogenase Kinase (PDK; PDK) Inhibitors</td>
<td>Anti fungal agents, Antineoplastic Antibiotics, Antimalaria drugs</td>
</tr>
<tr>
<td>Preclinical</td>
<td>279383 279387 279389</td>
<td>Norvatis</td>
<td>Pyruvate Dehydrogenase Kinase (PDK; PDK) Inhibitors</td>
<td>Anti diabetes drugs</td>
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<td>Biologic Testing</td>
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<td>Astrazeneca</td>
<td>Pyruvate Dehydrogenase Kinase 2 (PDK; PDK) Inhibitors</td>
<td>Anti diabetes drugs</td>
</tr>
</tbody>
</table>
PDK4 Inhibitor

PDK4 inhibitor Synthesis

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