Effect of PDK activation and PDH flux in Metabolic Syndrome ;

New Therapeutic Target of Metabolic syndrome and Cancer

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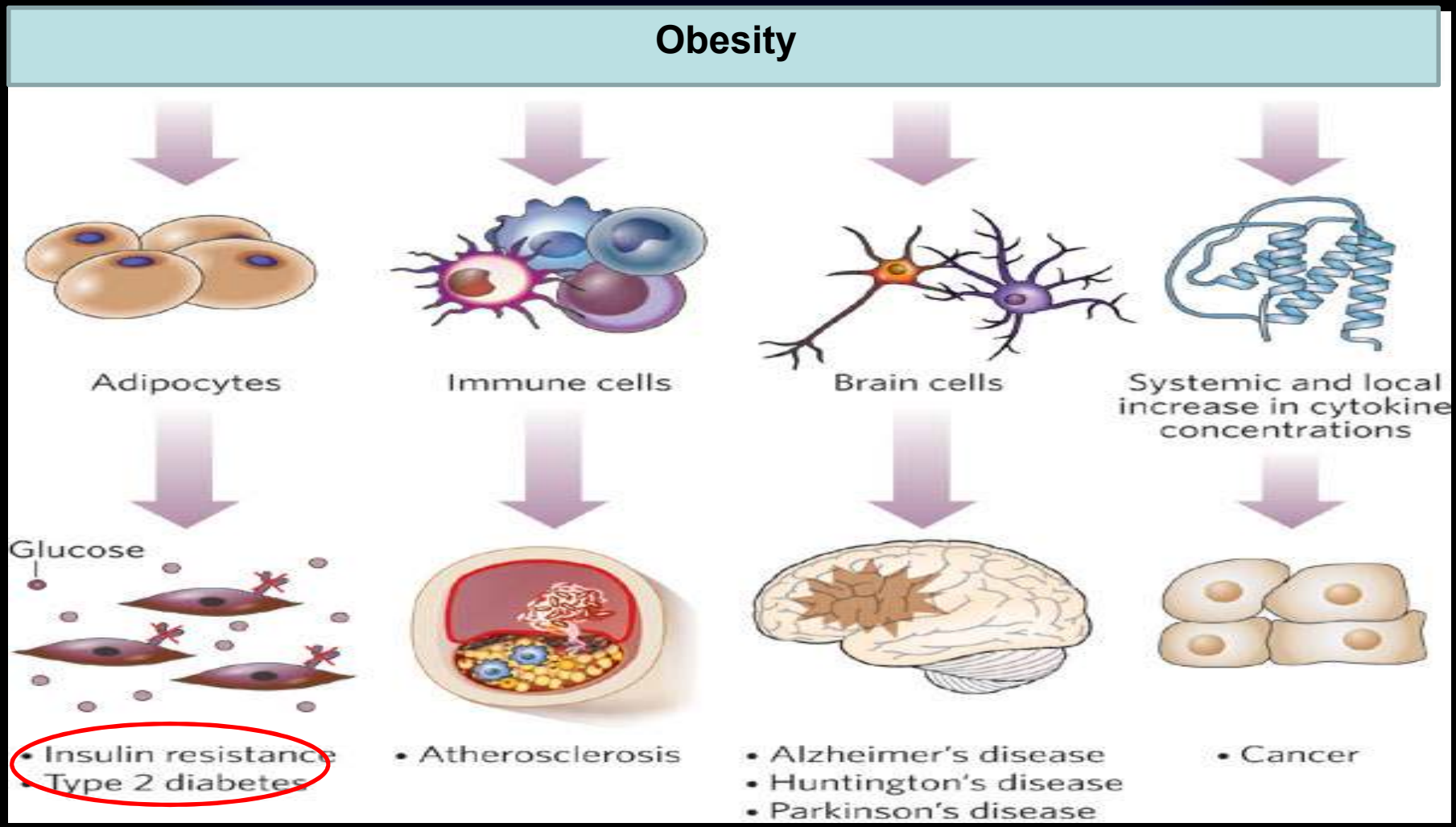
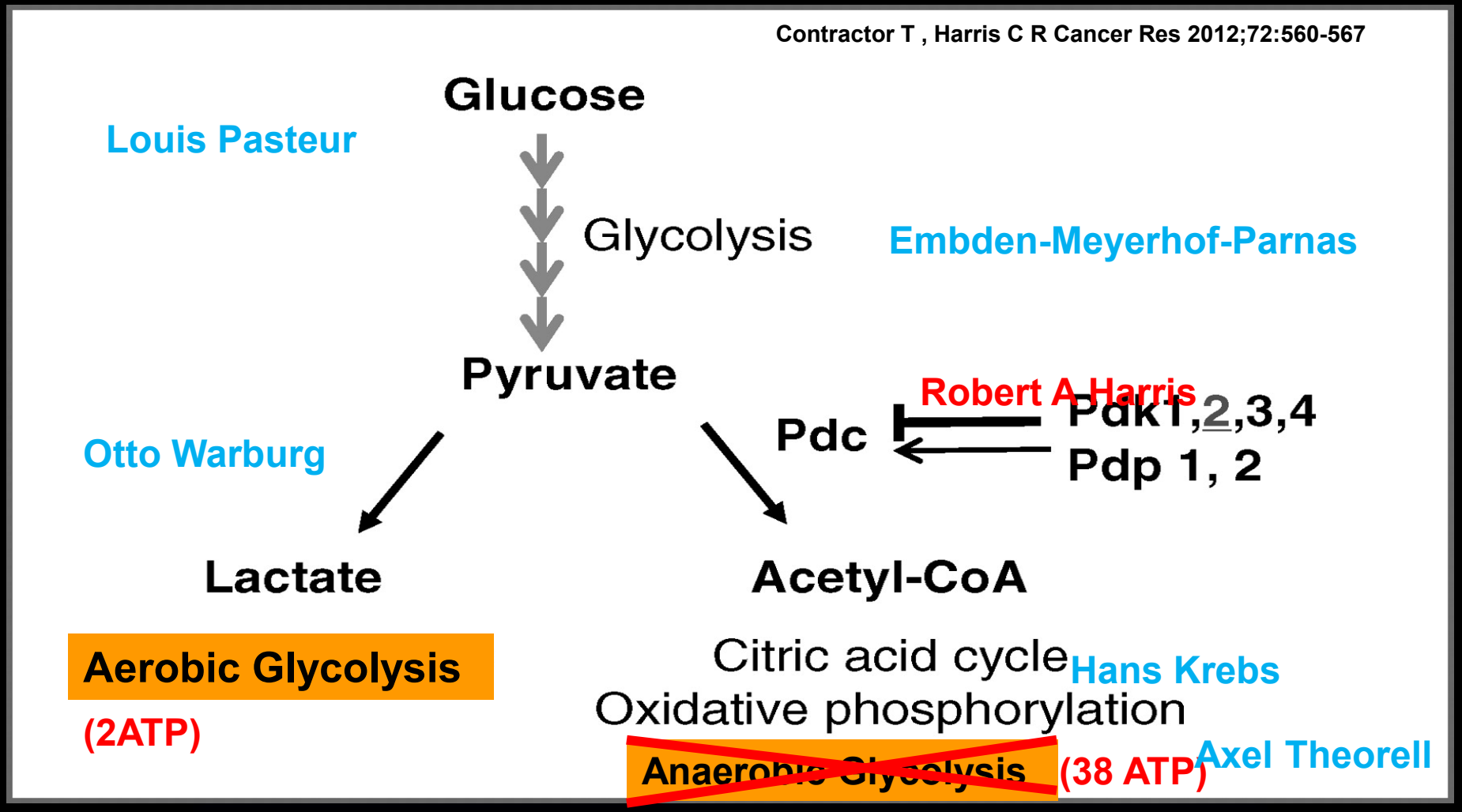
Obesity is linked to the development of many chronic diseases



metaflammation

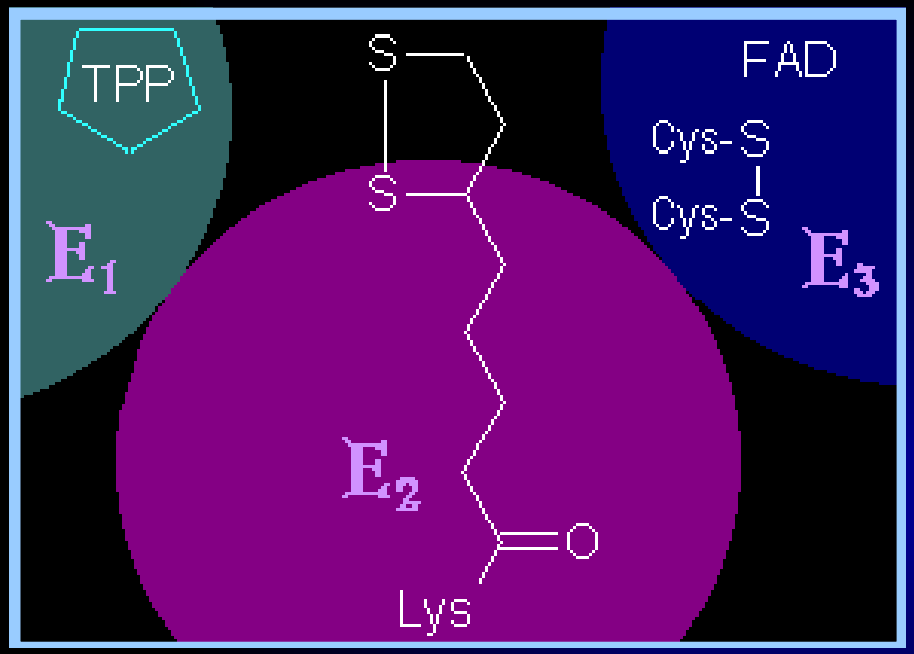
PDC is Main Gate of Oxidative Phosphorylation.



"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



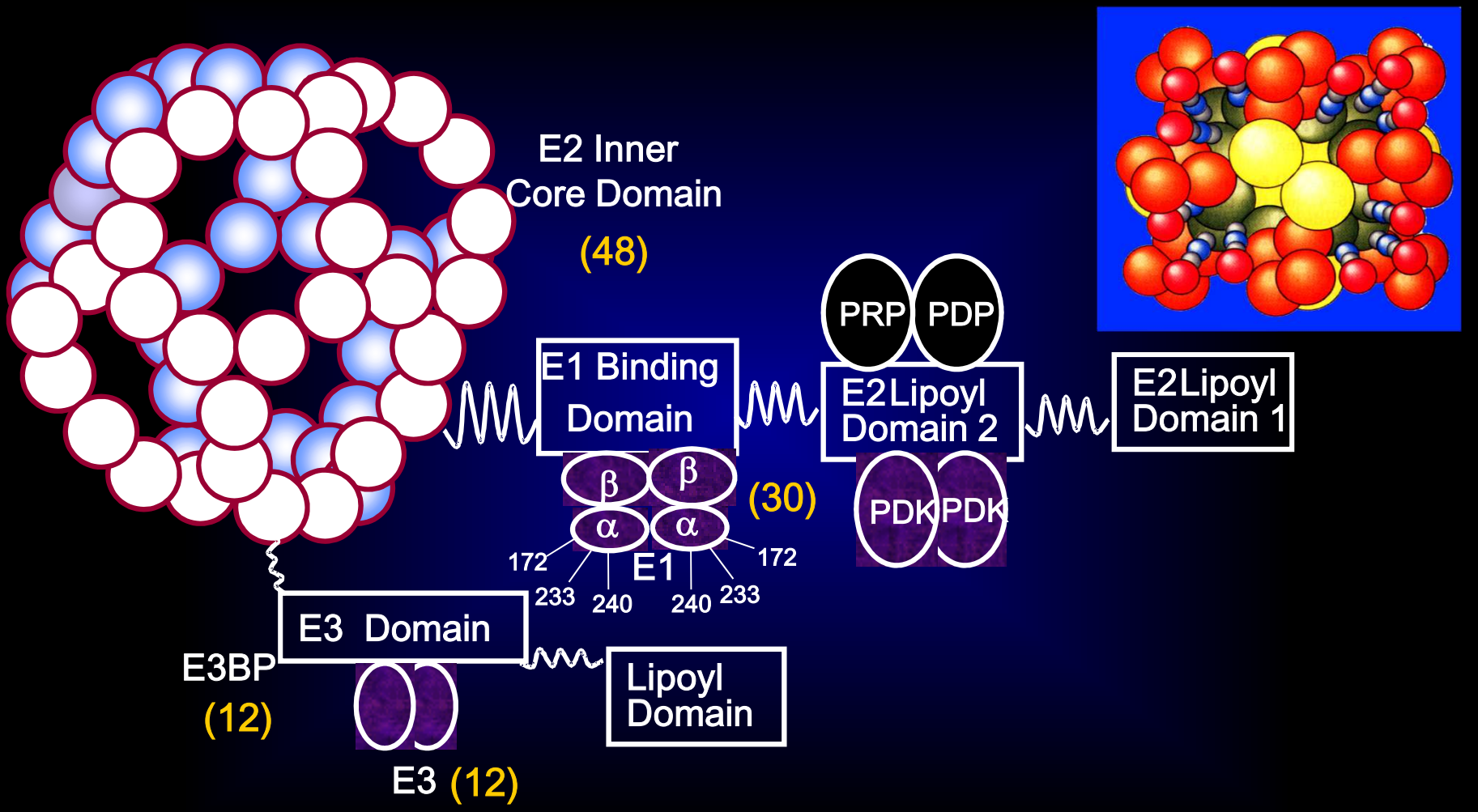
- **PDH :** Large complex containing many copies of 3 enzymes, $\mathbf{E_1}$, $\mathbf{E_2}$, & $\mathbf{E_3}$.
- The inner **core** of PDH is an **icosahedral** st. consist of **60** copies of $\mathbf{E_2}$.

- At the **periphery** of the complex are:

- 30 copies of $\mathbf{E_1}$ (itself a tetramer with subunits a_2b_2).

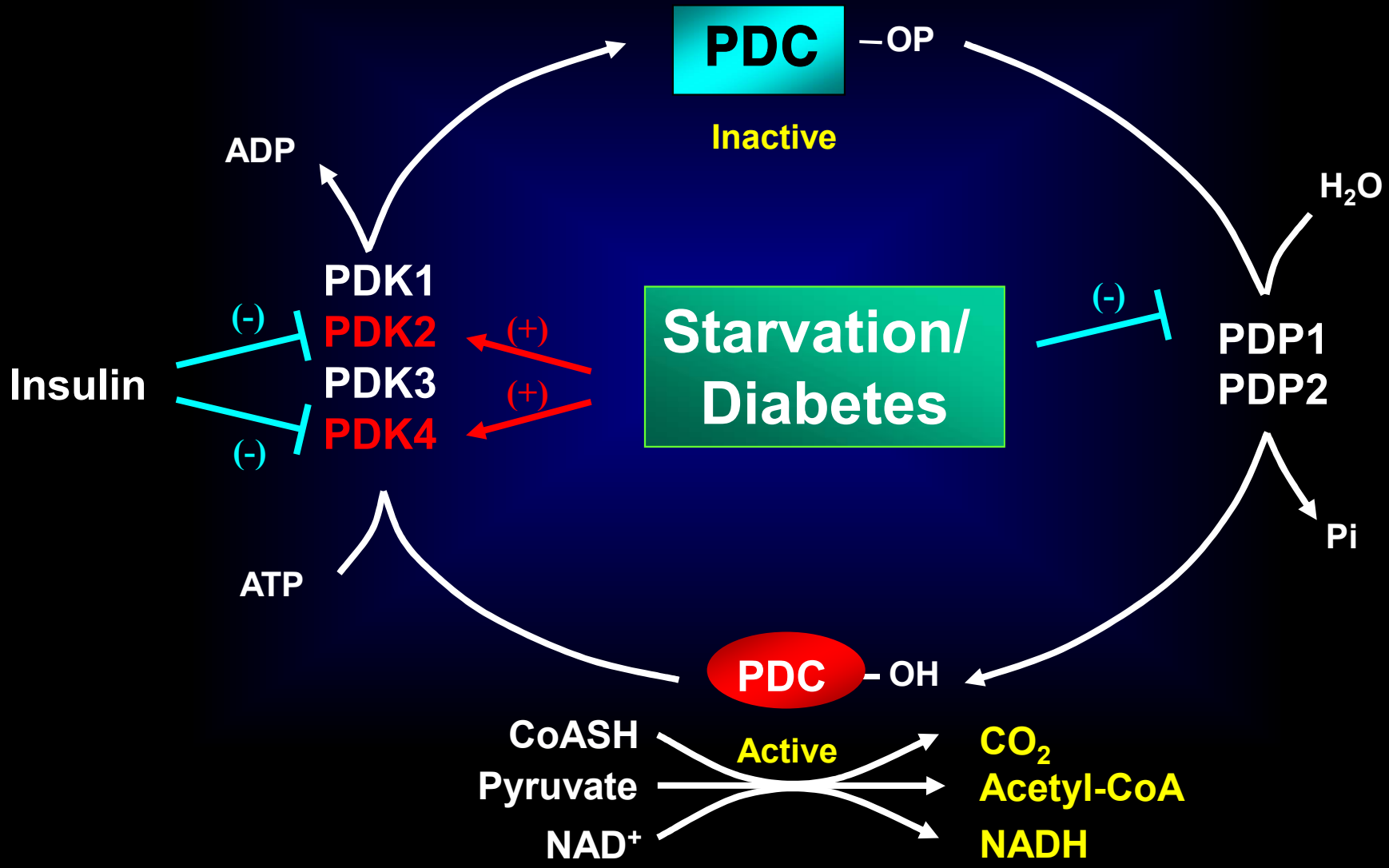
- 12 copies of $\mathbf{E_3}$ (a homodimer), plus **12** copies of an $\mathbf{E_3}$ **binding protein** that links E_3 to E_2.

PDH Complex Structure



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

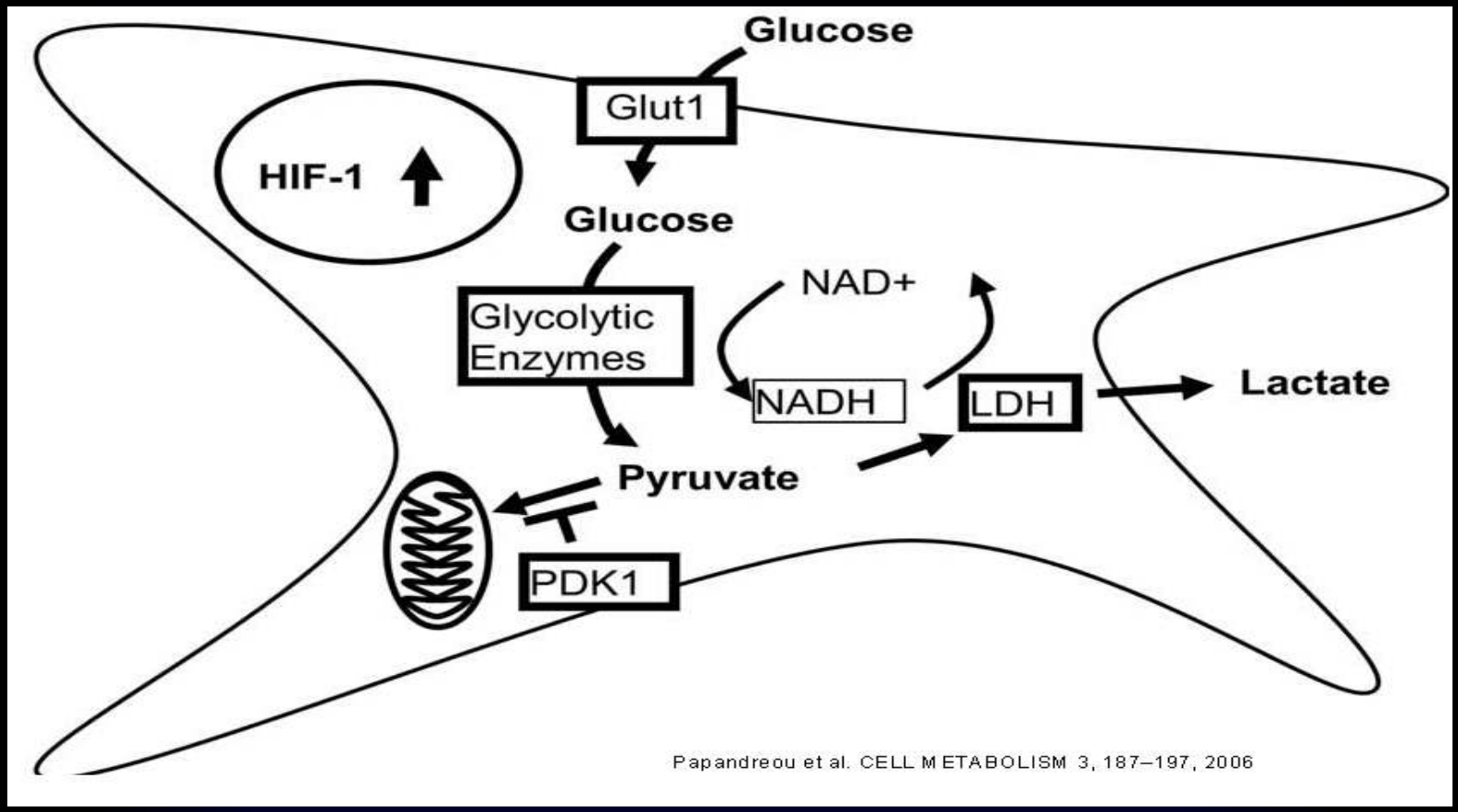


PDK in Cancer Cells

PDK in Metabolic Syndrome(Obesity, DM)

PDK in Vascular Calcification

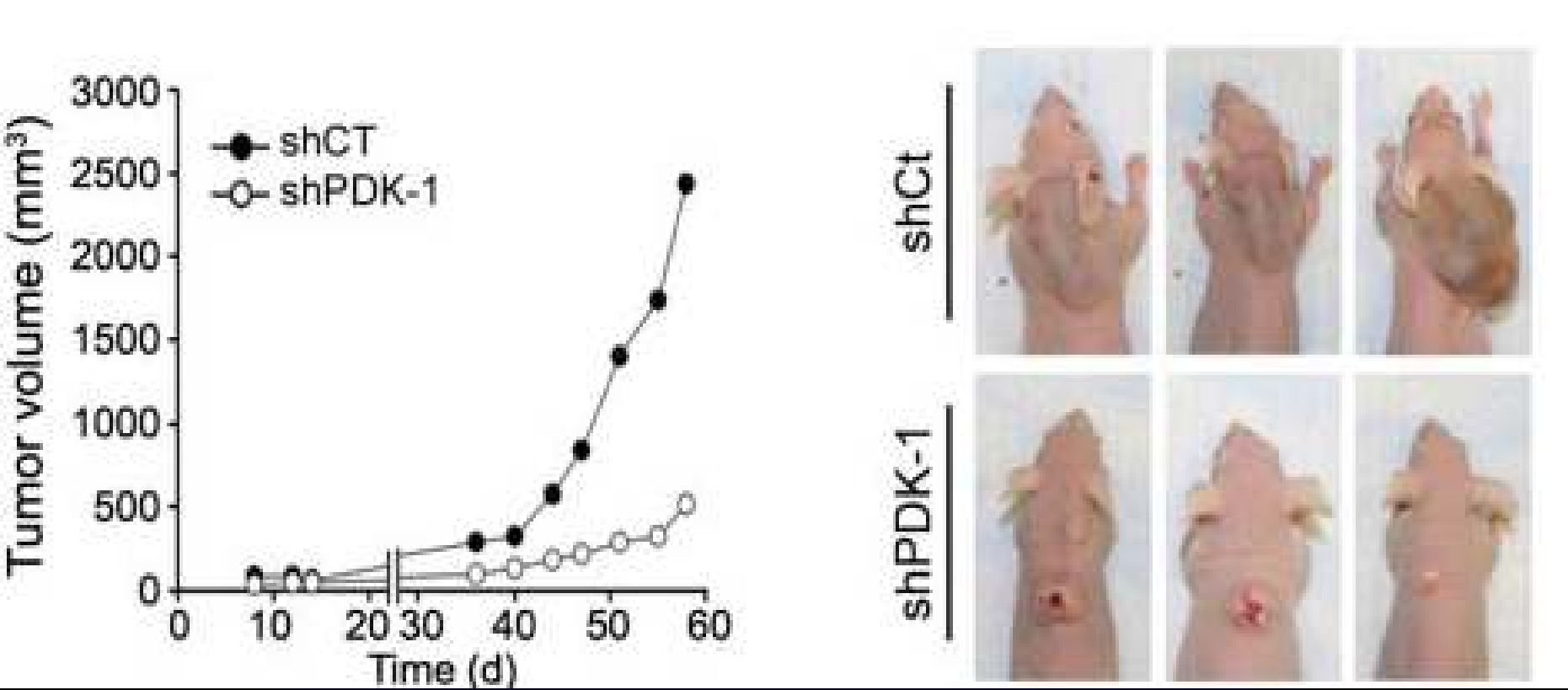
PDK in cancer cells



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

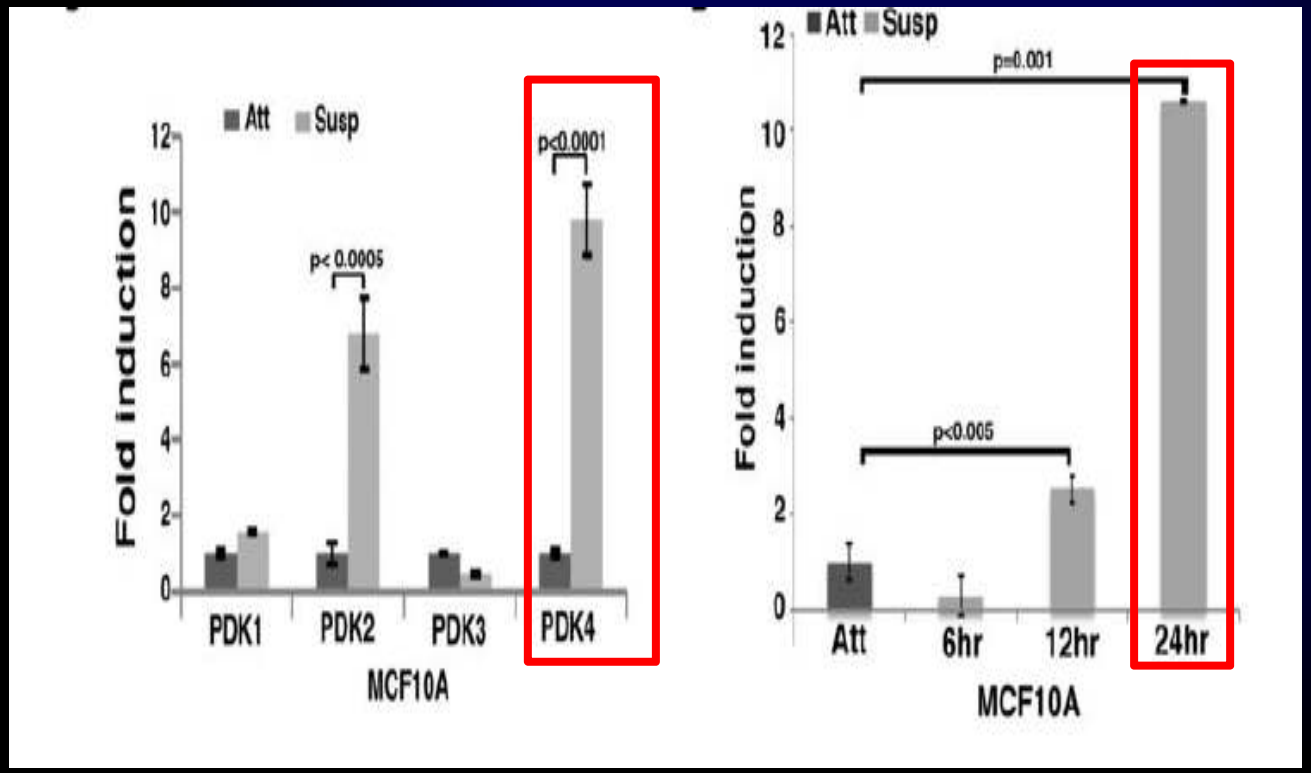
Effect of PDK1 knockdown on cancer growth

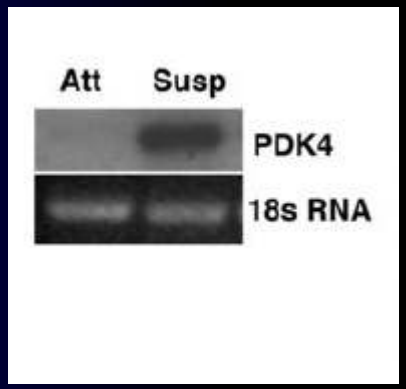


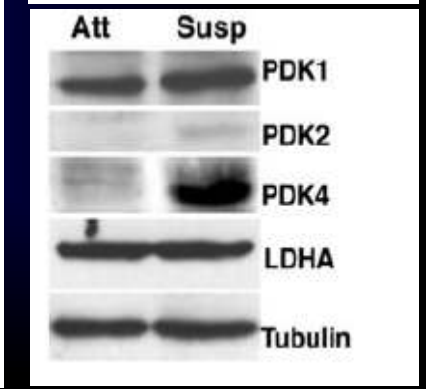
McFate T. *et.al.*, J. Biol. Chem., 283:22700 (2008)

PDK in cancer cells

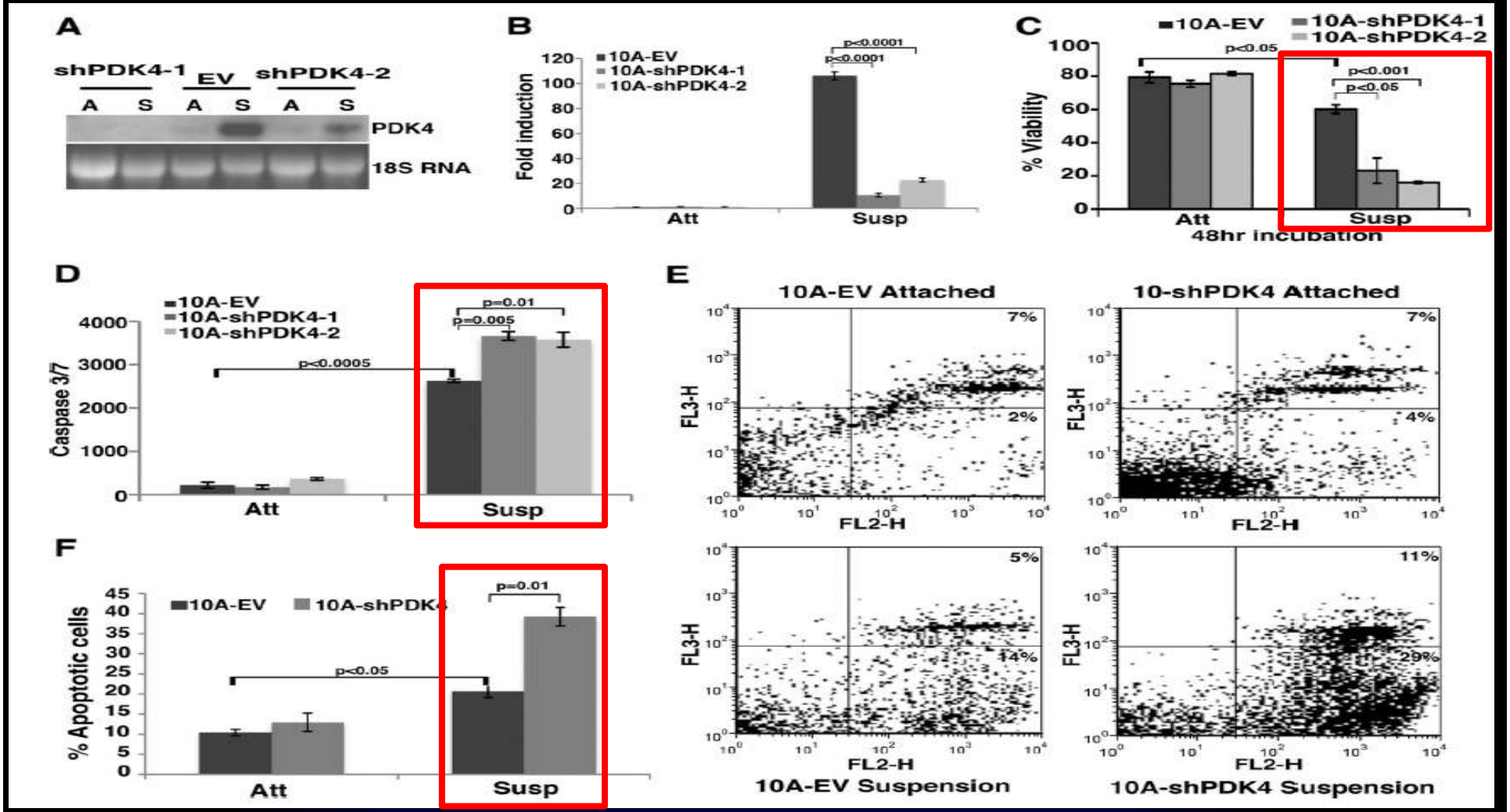
- PDK4 is overexpressed in a subset of human cancers and contributes to anoikis resistance in cancer cells
- Anoikis (Matrix detachment-induced apoptosis)







Sushama, et. al.Mol.Cell.Biol. 2012, *32(10): 1893.*



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

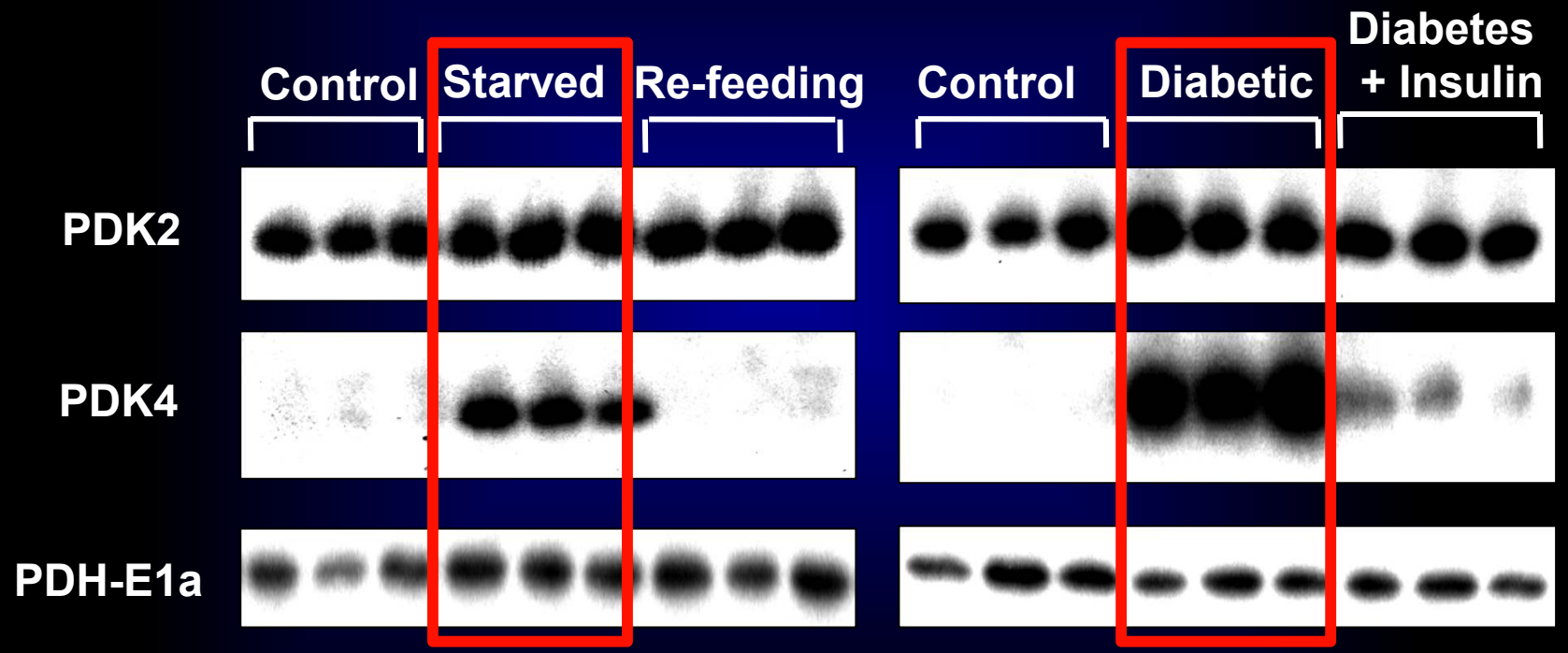
PDK in Metabolic Syndrome(Obesity, DM)

PDK in Vascular Calcification

(Question 1)

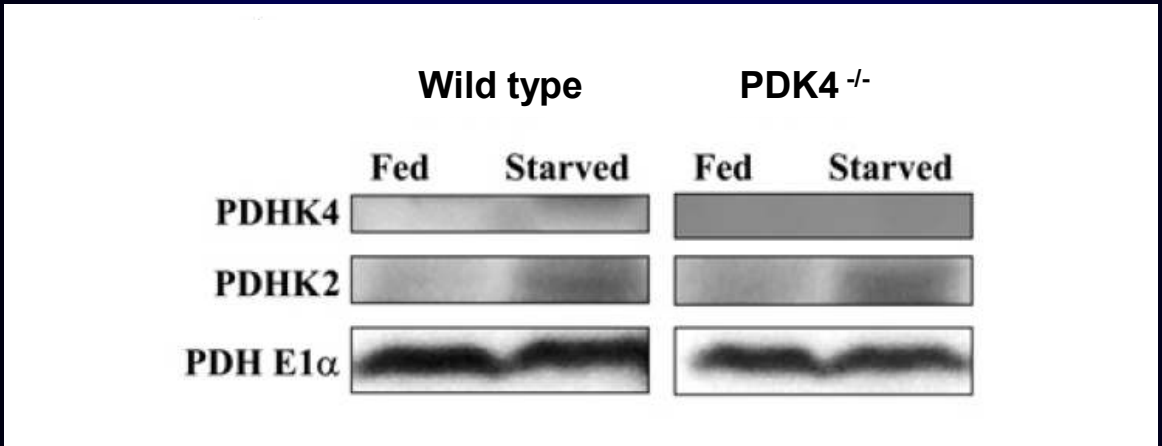
What is the Role of PDK4/2 on blood Glucose ?

Effect of Starvation and Diabetes on PDK2 and PDK4 Protein in Rat skeletal muscle



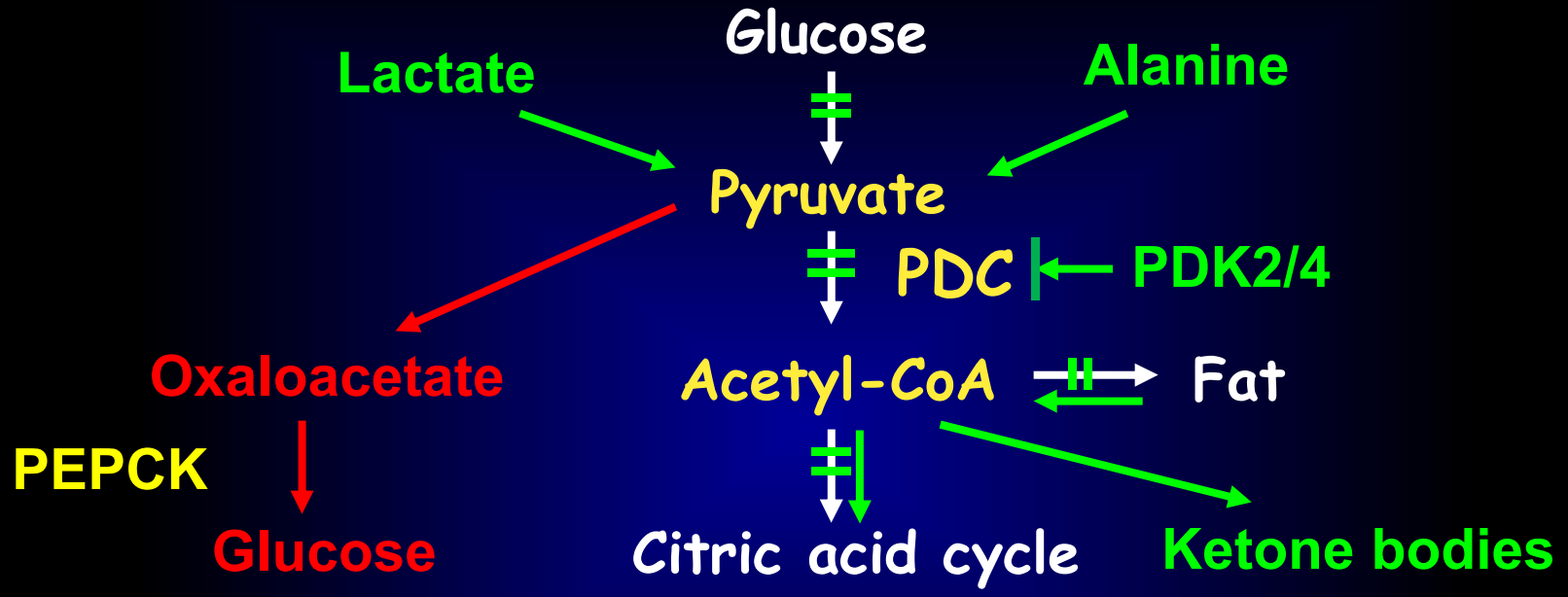
Wu et al. Diabetes (1999) 48: 1593-9

Effect of Starvation on PDK2 and PDK4 Protein in Liver of wild and PDK4$^{-/-}$ Mice



Jeoung, et. al. Biochem. J. (2006) 397:417-425

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

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(Question 2)

Does knocking out PDK4 decrease blood glucose level?
And how?

Blood Levels of Glucose in WT and KO Mice

Metabolic State	Blood from:	
	$PDK4^{+/+}$ mice	$PDK4^{-/-}$ mice
Fed (mg/dL)	140 ± 8	133 ± 10
Fasted overnight (mg/dL)	64 ± 3	45 ± 1*
Starved 48 hours (mg/dL)	139 ± 5	58 ± 3*

*P<0.01

Jeoung, et. al. Biochem. J. (2006) 397:417-425

Blood Levels of Three Carbon Compounds in WT and KO Mice

Measurements	Blood from:	
	$PDK4^{+/+}$ mice	$PDK4^{-/-}$ mice
Lactate (mmol/l)	1.93 ± 0.15	1.10 ± 0.10*
Pyruvate (mmol/l)	0.07 ± 0.01	0.03 ± 0.01*
Alanine (mmol/l)	0.26 ± 0.01	0.18 ± 0.01*

*P<0.01

Jeoung, et. al. Biochem. J. (2006) 397:417-425

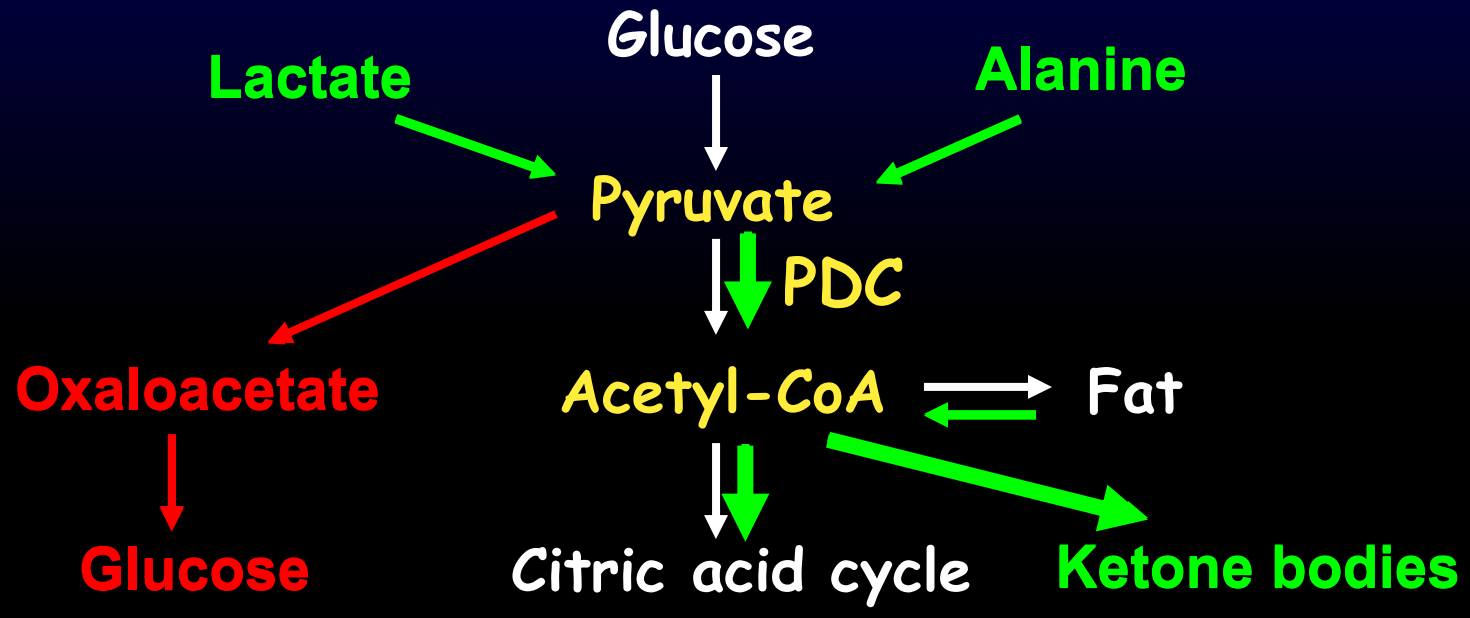
Effect of starvation on levels of lipids metabolites in wild-type and PDK4 KO mice

Measurements	Blood from:	
	Wild-type	PDK4$^{-/-}$
Free fatty acids (mmol/l)	1.44 ± 0.13	2.20 ± 0.07*
3-Hydroxybutyrate (mmol/l)	1.90 ± 0.60	6.38 ± 1.81*
Acetoacetate (mmol/l)	0.14 ± 0.04	1.28 ± 0.11*

Biochem. J. (2009) 423, 243–252

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

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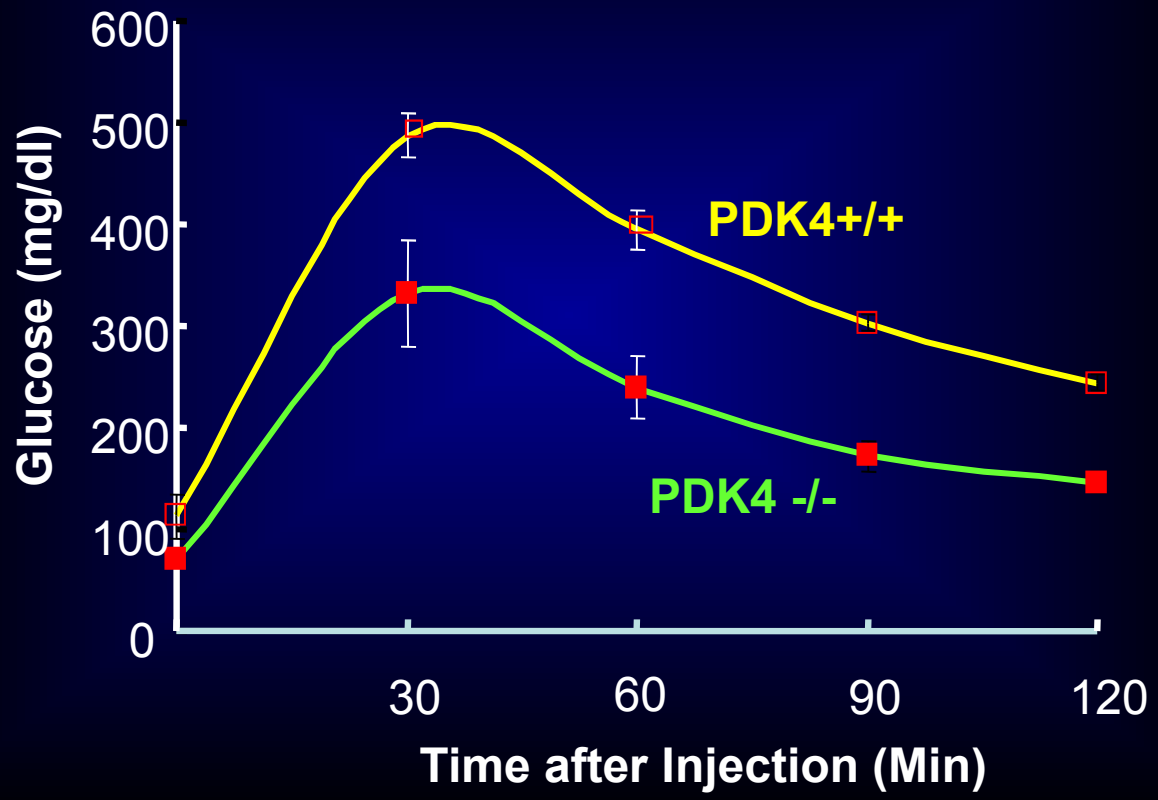
Glucose Tolerance Test on High Fat Fed Mice (n=5 mice/group)



Biochem. J. (2009) 423, 243–252

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

Genotype	Body weight	Epididymal fat pads
PDK4$^{+/+}$ (gram)	48.3 ± 1.8	3.59 ± 0.11
PDK4$^{-/-}$(gram)	41.1 ± 1.5*	2.68 ± 0.12*

N= 6 mice each group; *P < 0.05

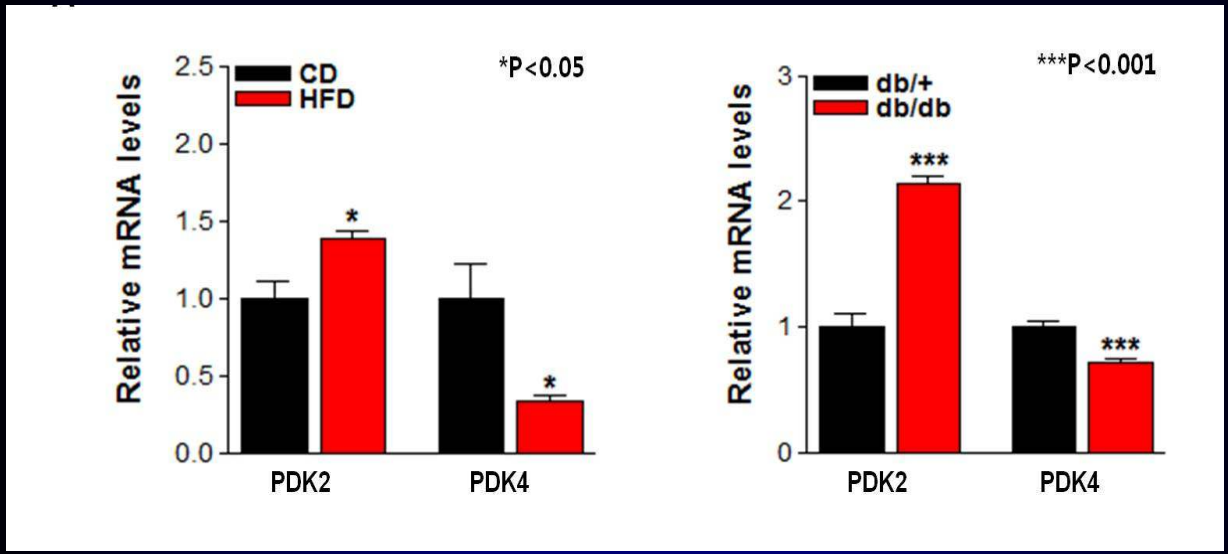
Biochem. J. (2009) 423, 243–252

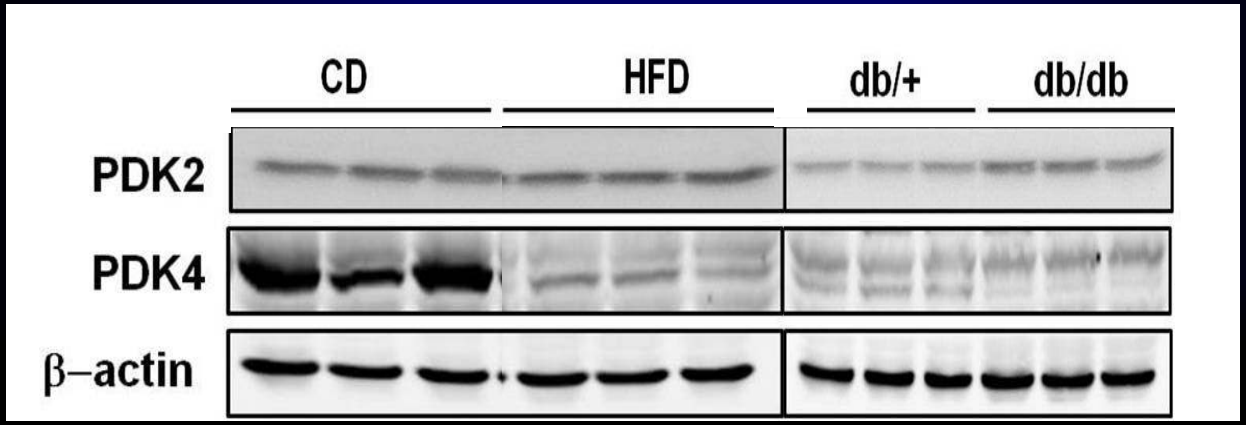
Summary(1)

- 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.

Effect of DIO and Diabetes on PDK2 and PDK4 Expression in Liver





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(Question 4)

What about PDK2?

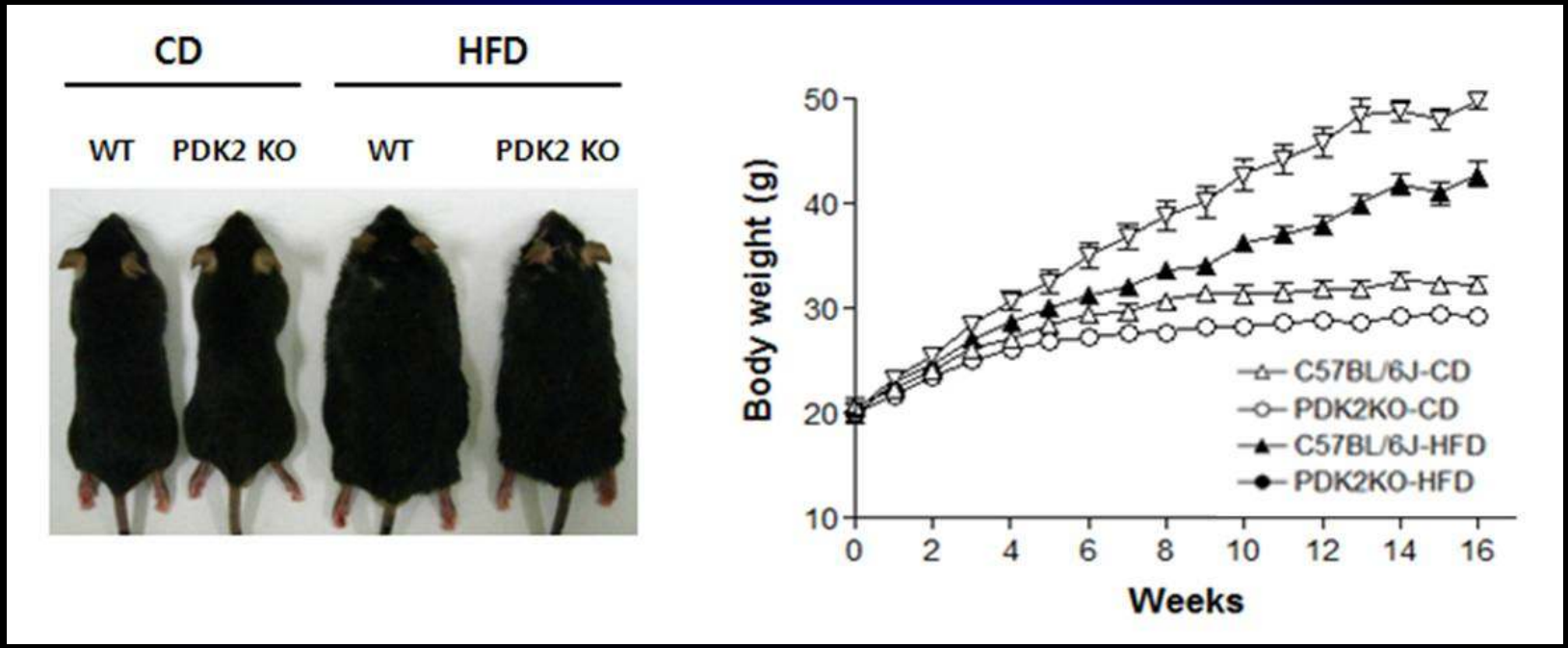
What about PDK2?

- 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$^{-/-}$ mice in diet induced obese (DIO) mice

Measurement†	Genotype	
	Wild-type	PDK2$^{-/-}$
Glucose (mg/dl)	179.8 ±8.8	▼ 152.7±7.9*
Lactate (mmol/L)	1.12± 0.10	0.93 ± 0.09
Pyruvate (mmol/L)	0.17 ± 0.01	0.15 ± 0.01
3-Hydroxybutyrate (mmol/L)	1.04 ± 0.12	▲ 1.64 ± 0.07***
Acetoacetate (mmol/L)	0.14 ± 0.02	▲ 0.23 ± 0.02*
Free fatty acids (mmol/L)	0.21 ± 0.02	0.18 ± 0.02

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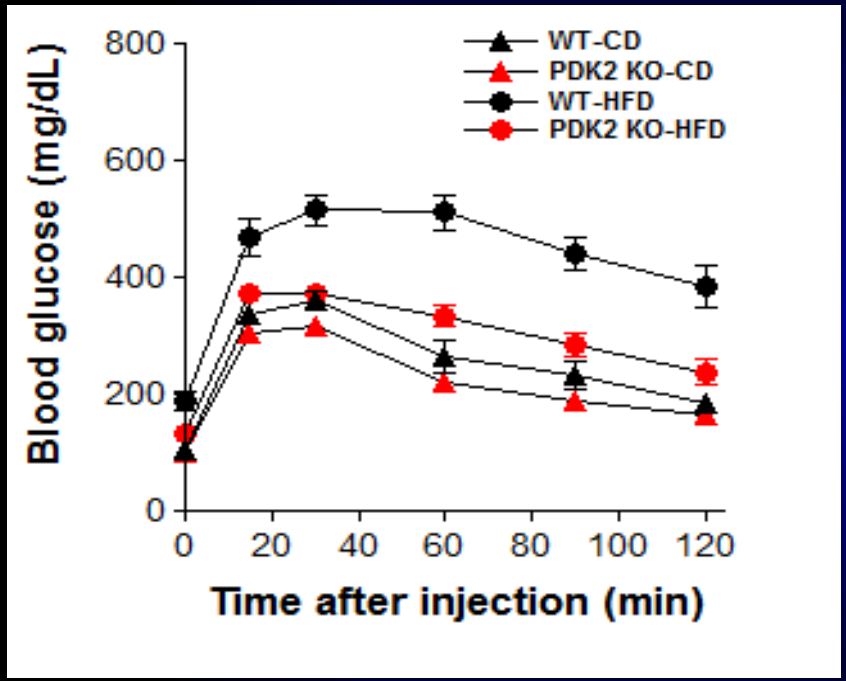
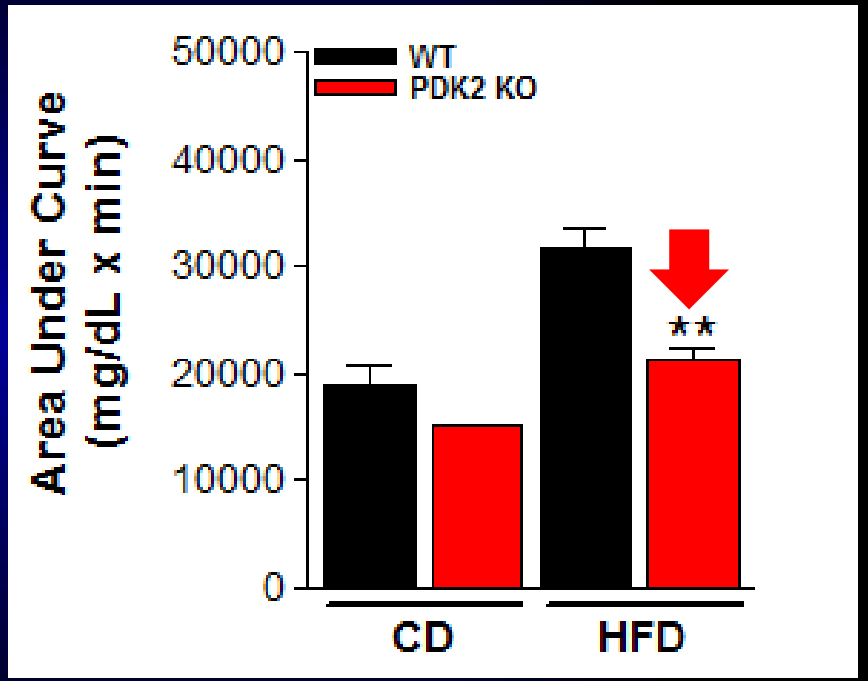
Glucose Tolerance Test of PDK2$^{-/-}$ mice in DIO





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Metabolic profiles of wild-type and PDK2$^{-/-}$ mice

Parameters	Control diet		High fat diet	
	wild-type	PDK2$^{-/-}$	wild-type	PDK2$^{-/-}$
Blood glucose (mg/dL)	94.5±0.5	92.8±2.9	179.8±8.8	152.7±7.9 *
Insulin (ng/ml)	1.3±0.1	0.9±0.4	4.8±0.4	1.8±0.4 ***
Total protein (g/dL)	5.4±0.1	5.0±0.1	5.6±0.1	5.2±0.1
Total cholesterol (mg/dL)	119.0±2.7	110.0±3.9	181.6±12.6	134.8±5.3 **
HDL (mg/dL)	64.0±1.0	54.5±3.2 #	62.5±2.2	63.3±2.4
LDL (mg/dL)	4.4±0.4	5.9±0.5	9.5±2.2	4.7±0.3 *
Triglycerides (mg/dL)	51.4±3.1	34.3±1.1 ##	41.1±3.4	49.3±3.6
Free fatty acid (mM)	0.12±0.02	0.29±0.03 #	0.21±0.02	0.18±0.02
Adiponectin (mg/dL)	11.7±1.1	11.9±0.8	12.3±1.0	15.6±1.1 *
Leptin (ng/ml)	2.0±0.2	2.7±1.9	190.5±1.6	65.0±16.5 ***
Resistin (ng/ml)	4.8±0.6	3.5±0.4	8.7±0.2	8.6±0.1
Pyruvate (mM)	017±0.02	0.14±0.03	0.17±0.01	0.15±0.02
Lactate (mM)	1.32±0.02	1.25±0.02 #	1.12±0.10	0.93±0.09

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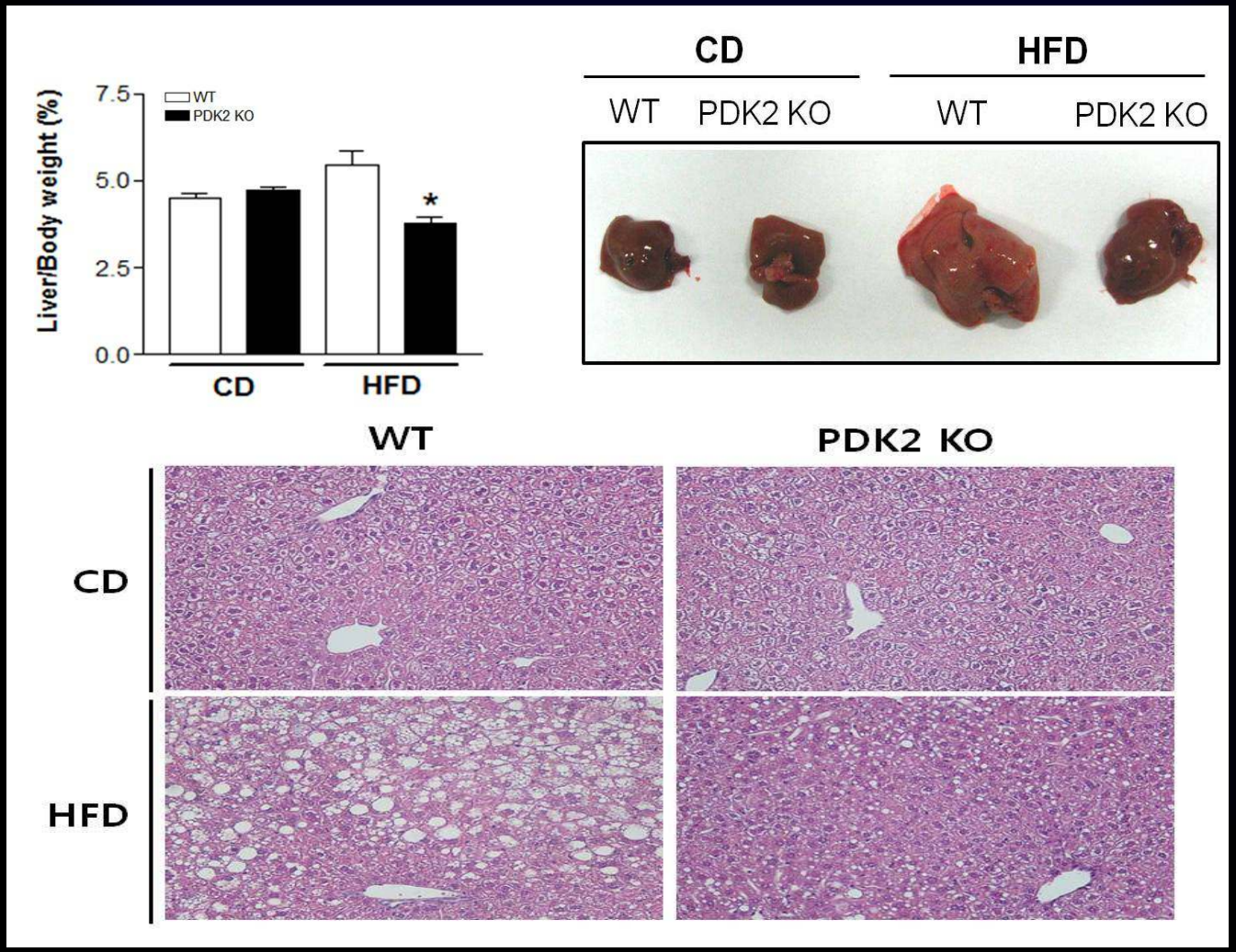
Effect of PDK2 deficiency on hepatic steatosis induced by the high fat diet



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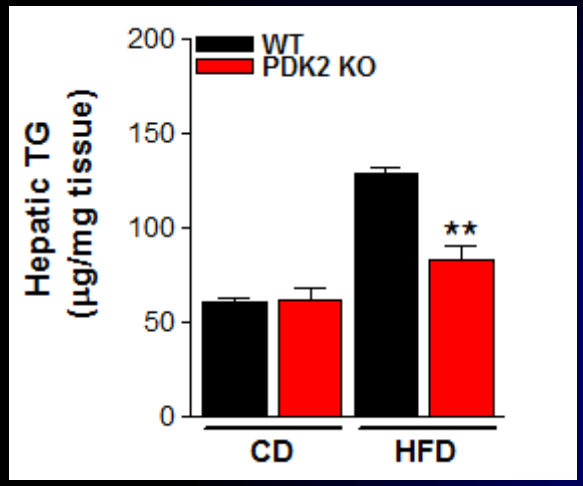

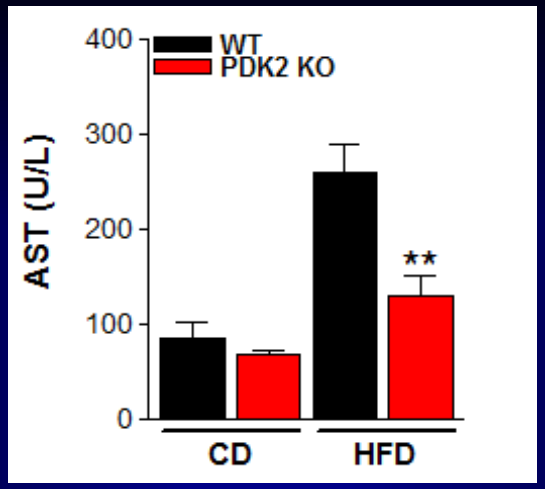

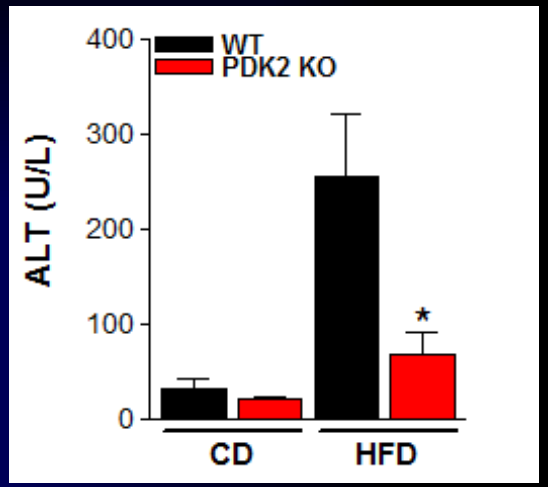

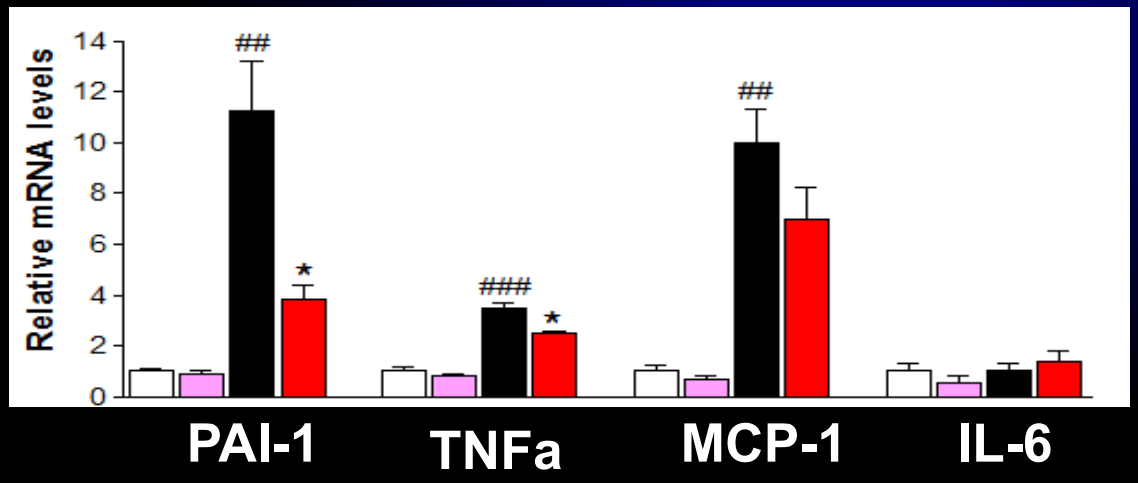

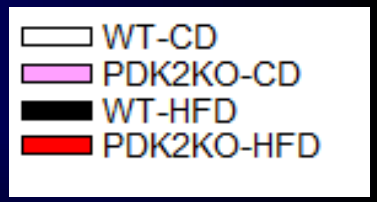

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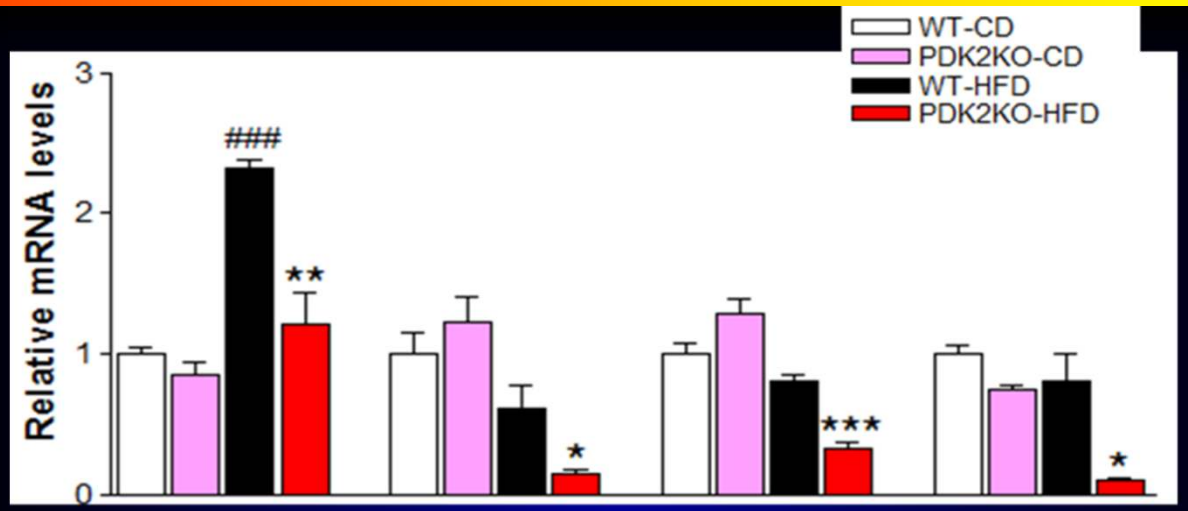
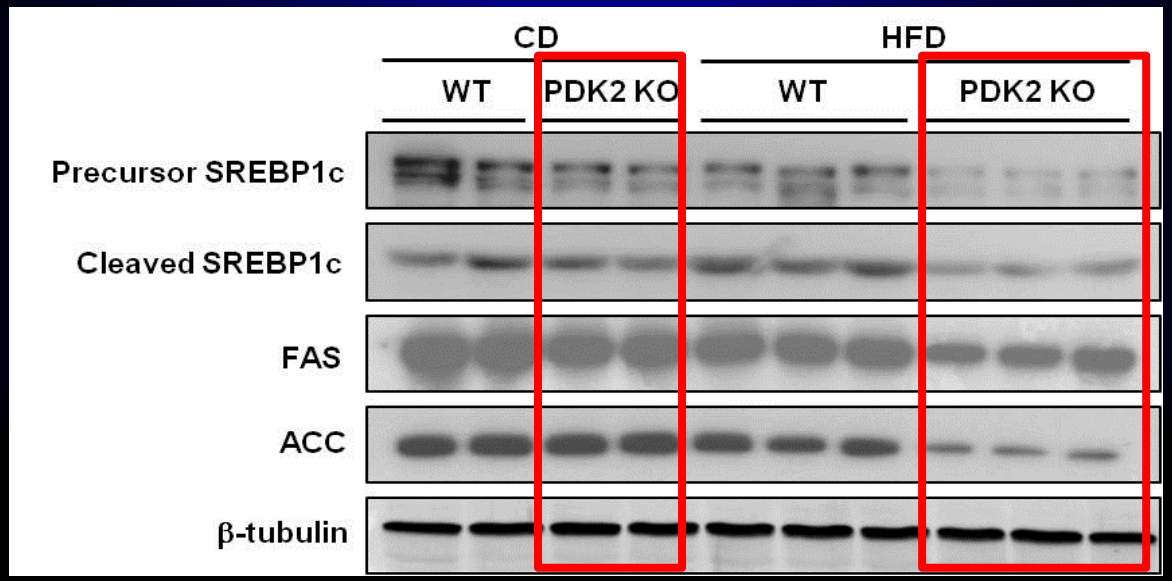
PDK2 deficiency suppresses fat synthesis





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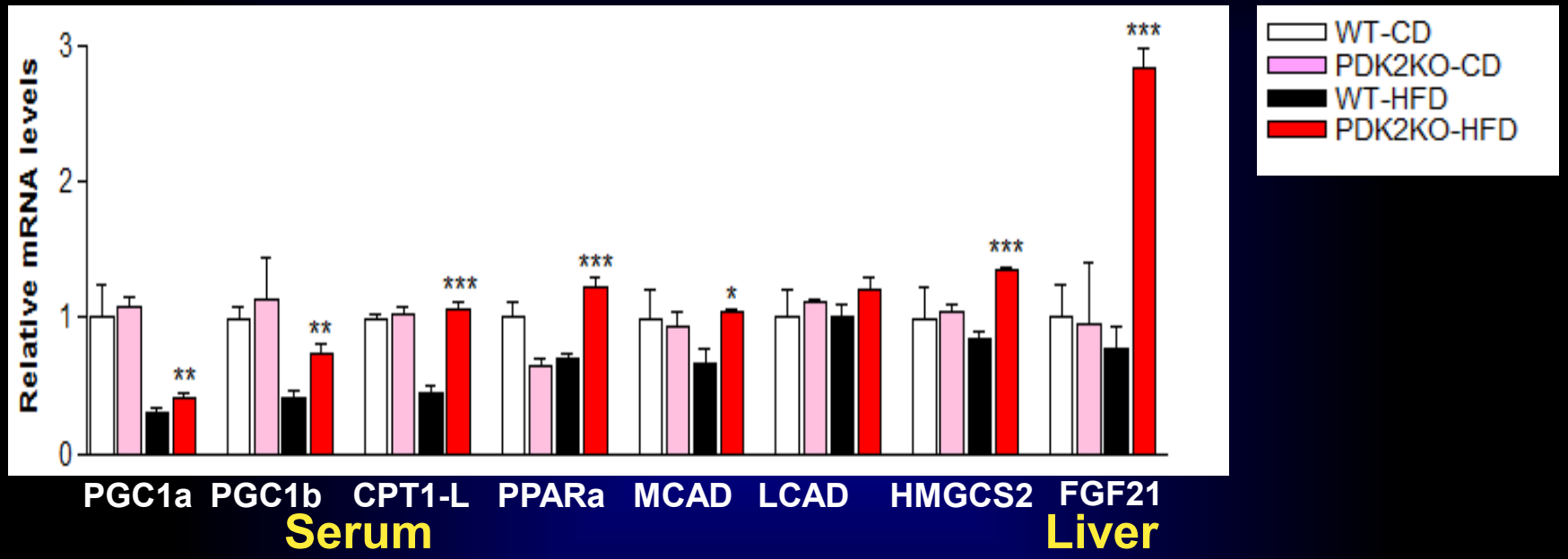
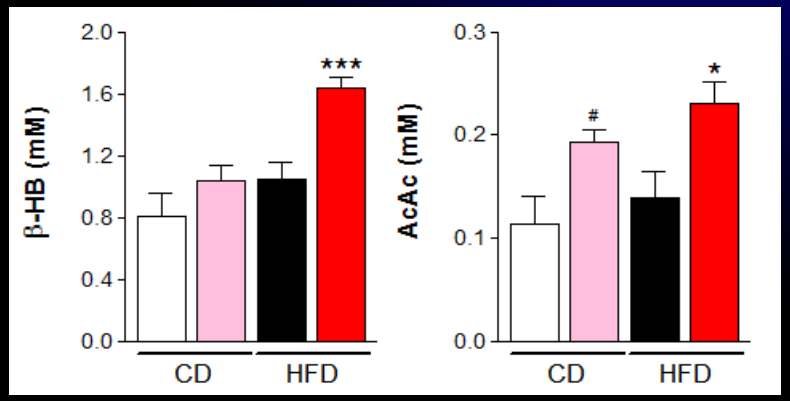
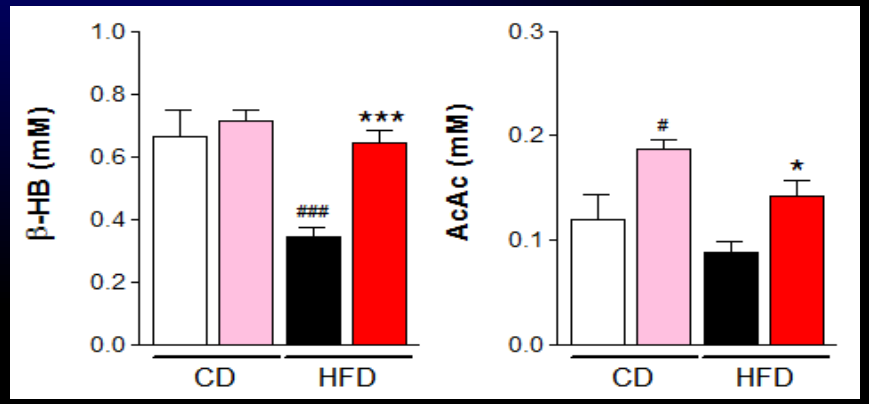
PDK2 deficiency activates FA oxidation and ketogenesis







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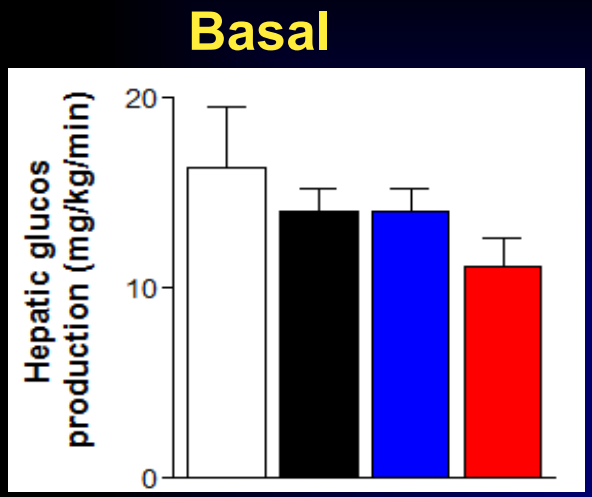
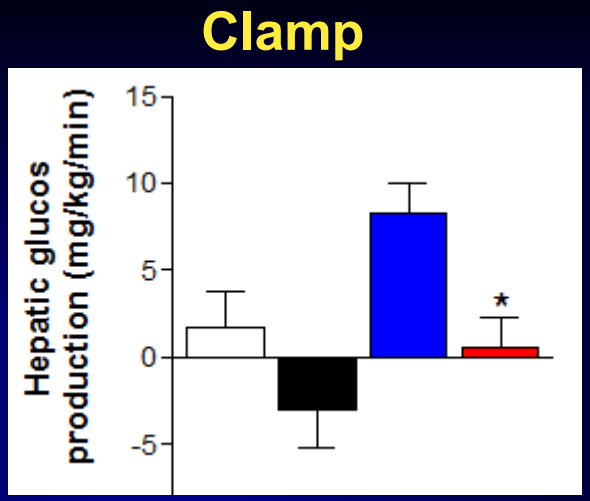
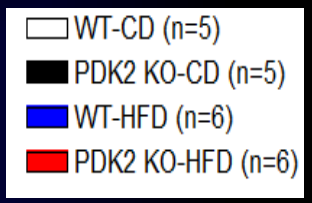
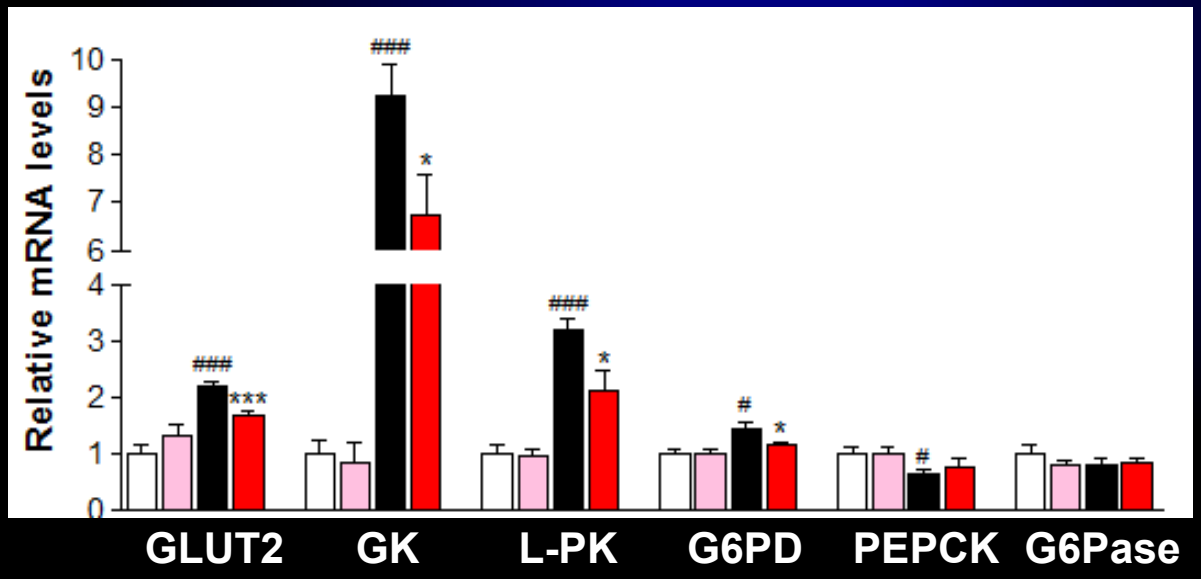
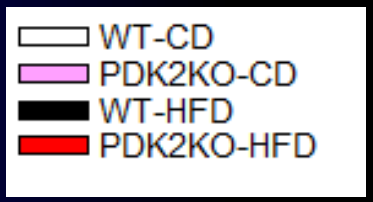
Improvement of hepatic glucose homeostasis in PDK2 deficiency mice











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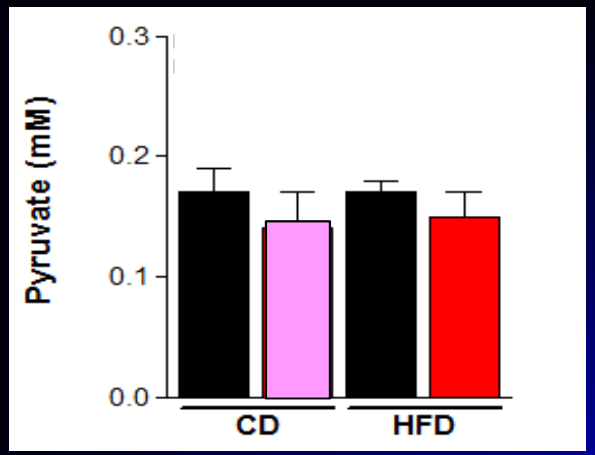
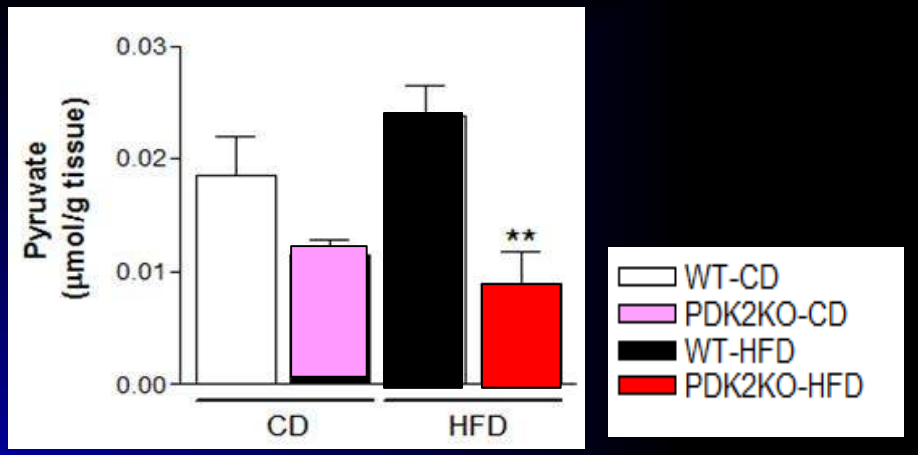
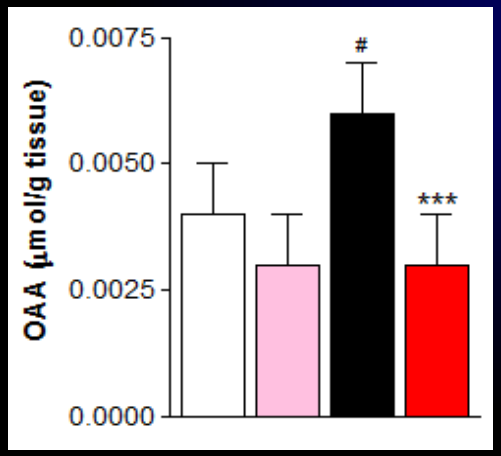
Reductions of the anaplerotic influx and the TCA cycle intermediates in PDK2 deficiency mice



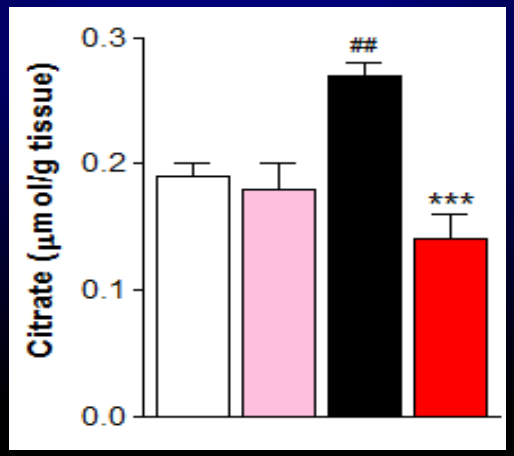
Serum



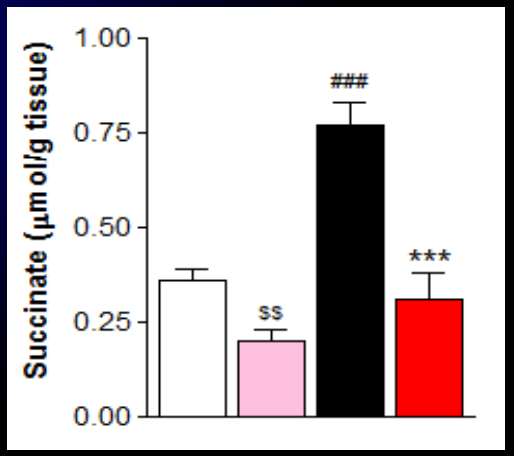
Liver



Oxaloacetate



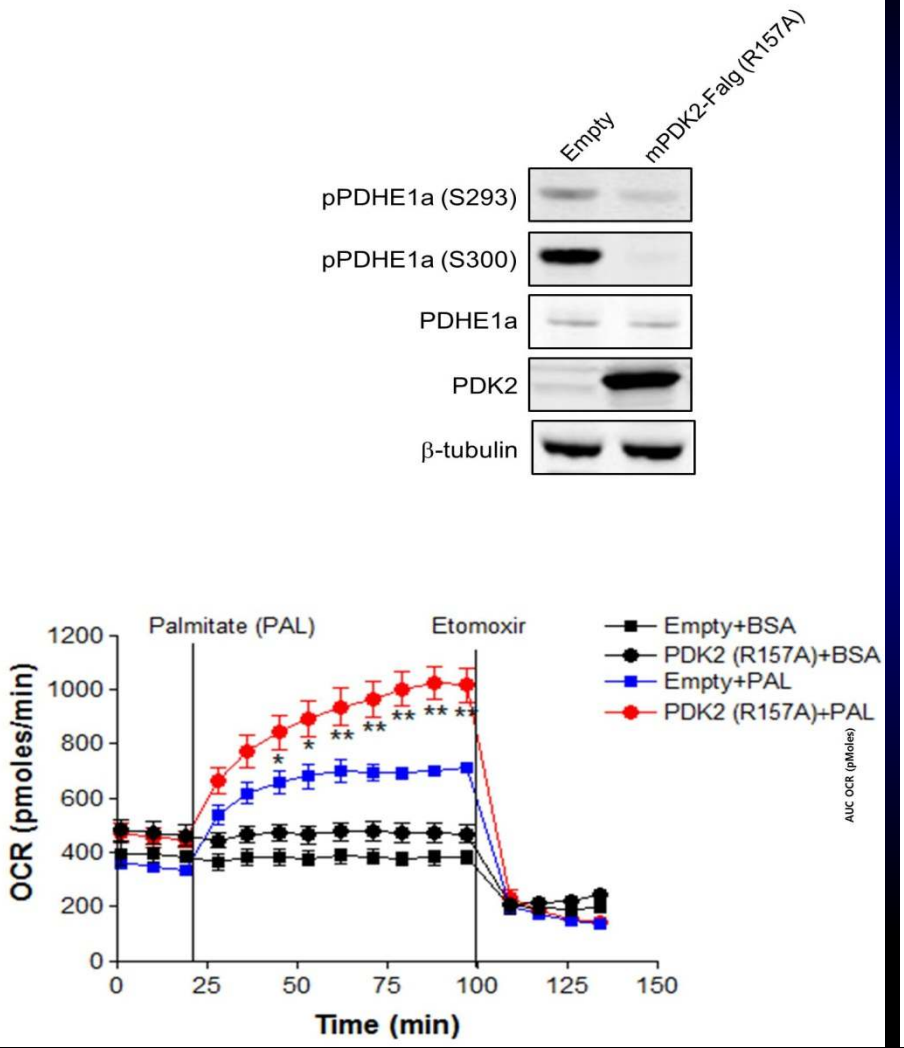
Citrate

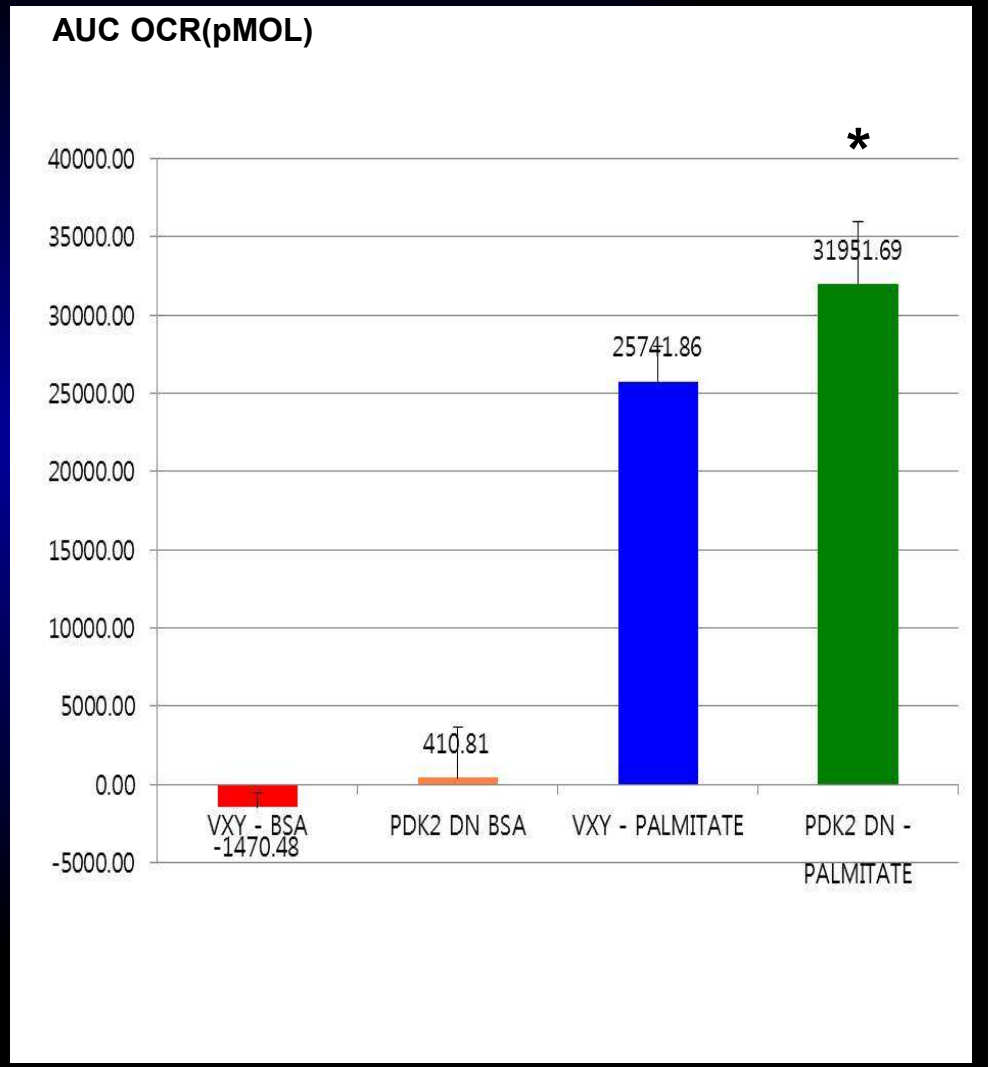


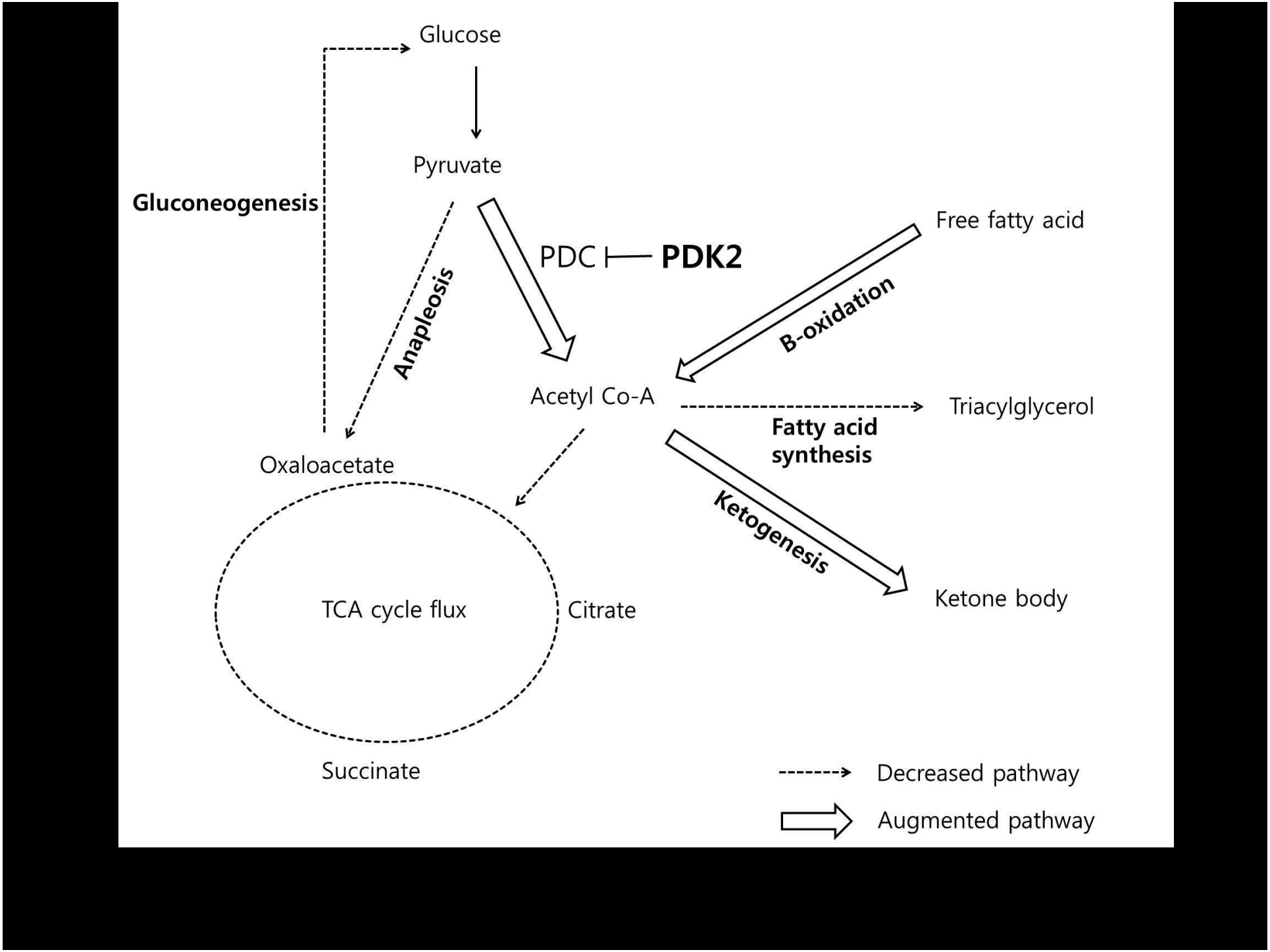
Succinate

Lab of endocrinology

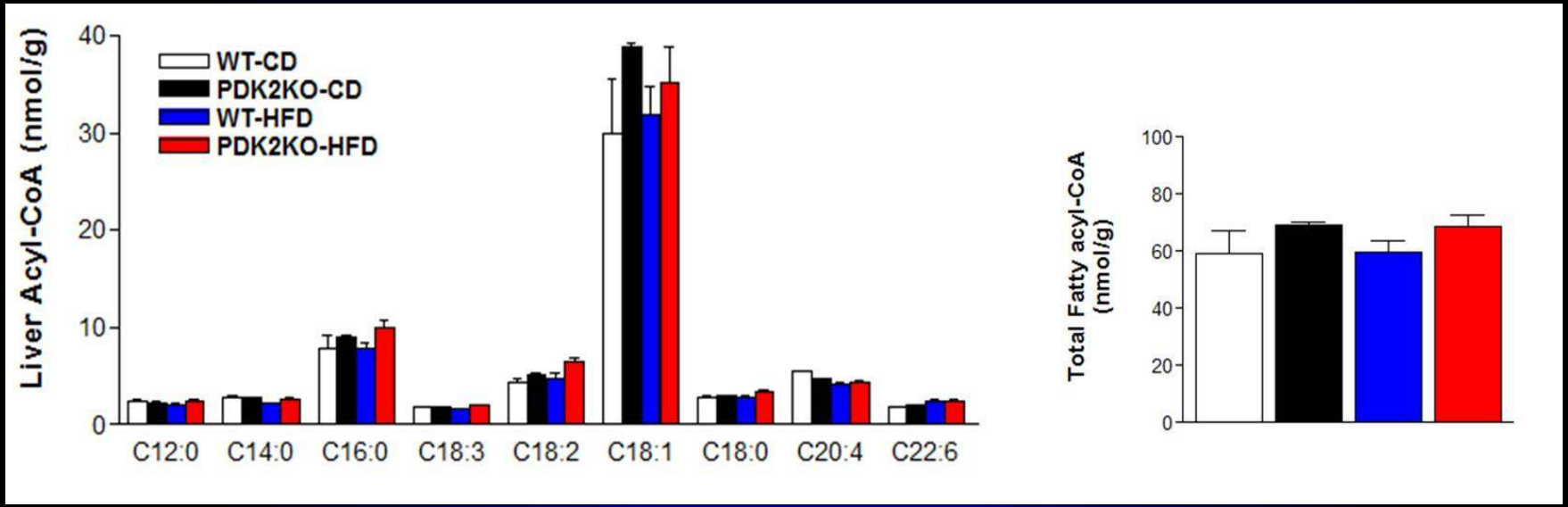
PDK2 Knock-Down Increased Beta-oxidation by XF-analyzer

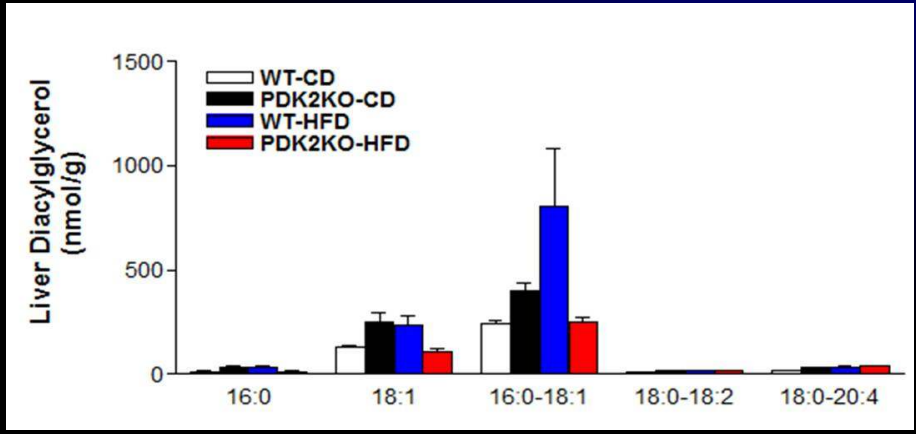


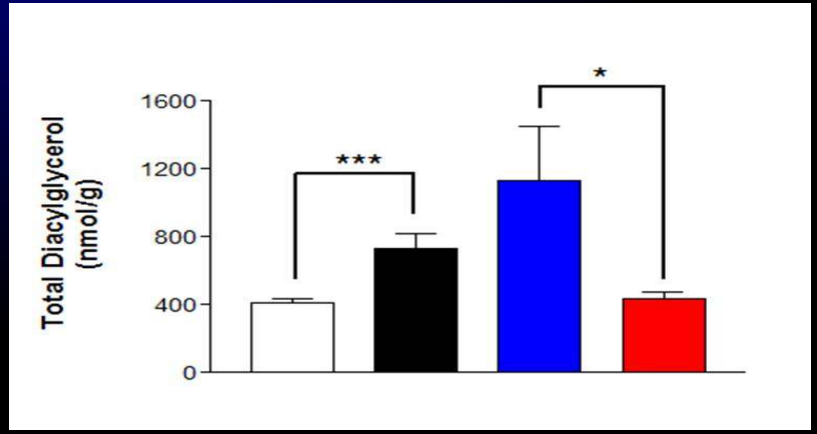



Glucose
Pyruvate
Gluconeogenesis
PDC
PDK2
Free fatty acid
B-oxidation
Anapleosis
Acetyl Co-A
Triacylglycerol
Fatty acid synthesis
Oxaloacetate
Ketogenesis
Ketone body
TCA cycle flux
Citrate
Succinate
Decreased pathway
Augmented pathway

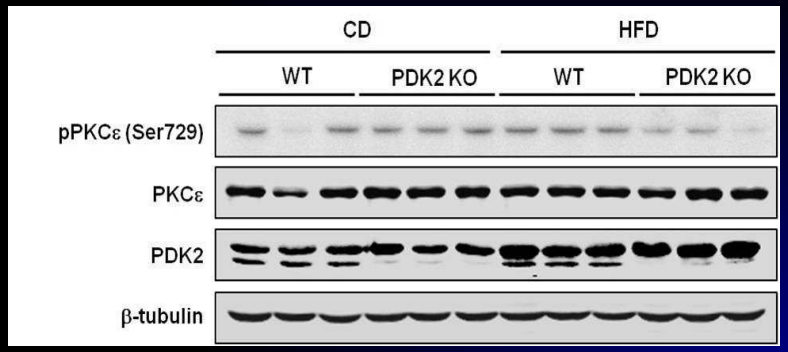
HFD-induced hepatic insulin resistance is ameliorated by PDK2 deficiency due to the decreased diacylglycerol (DAG)

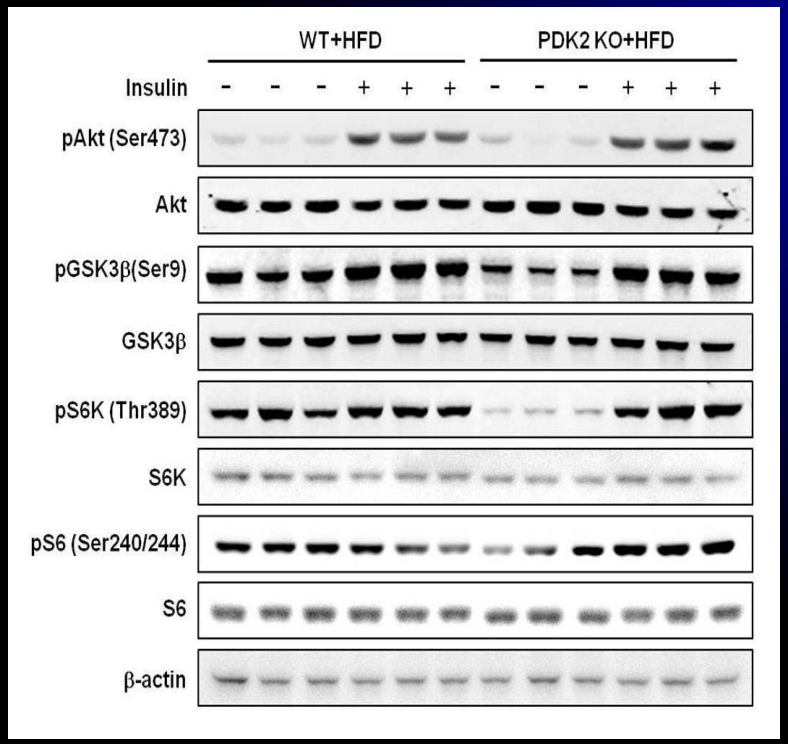


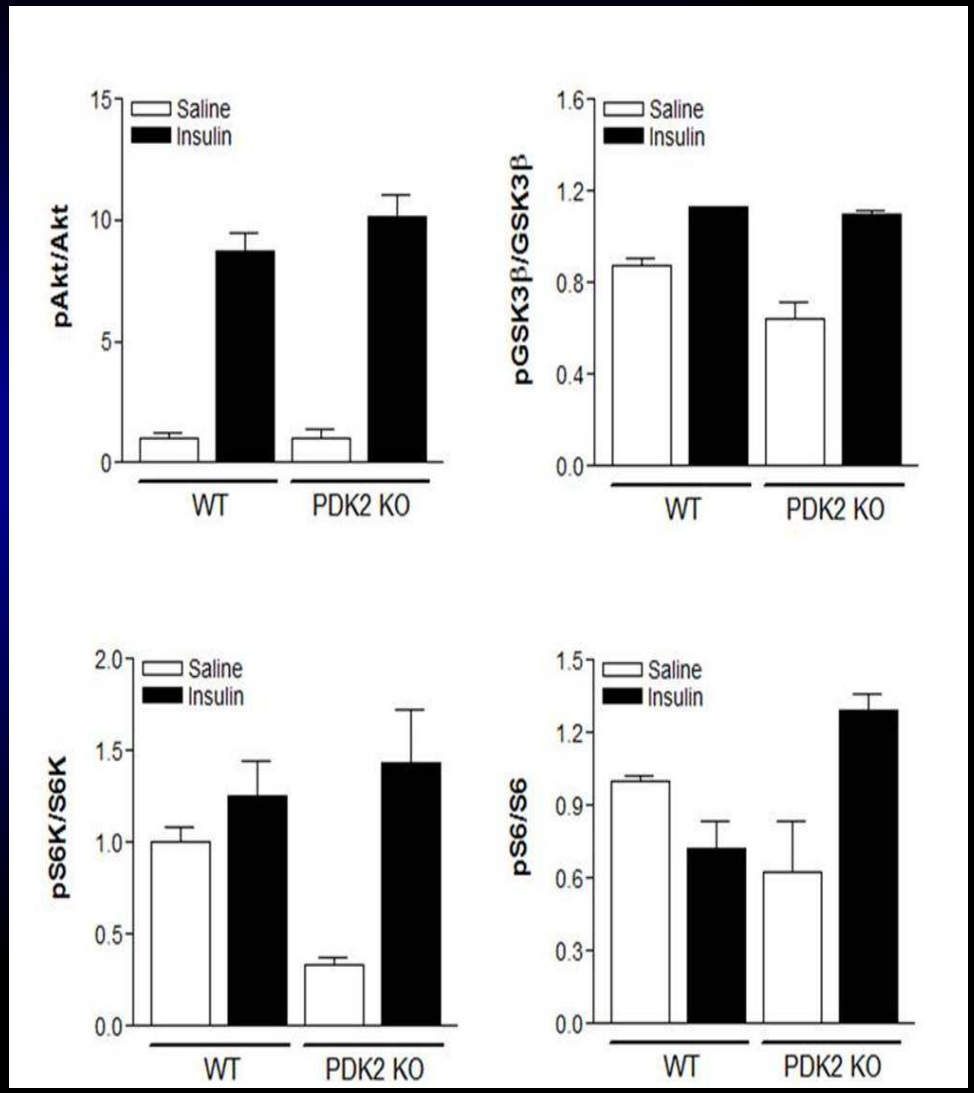




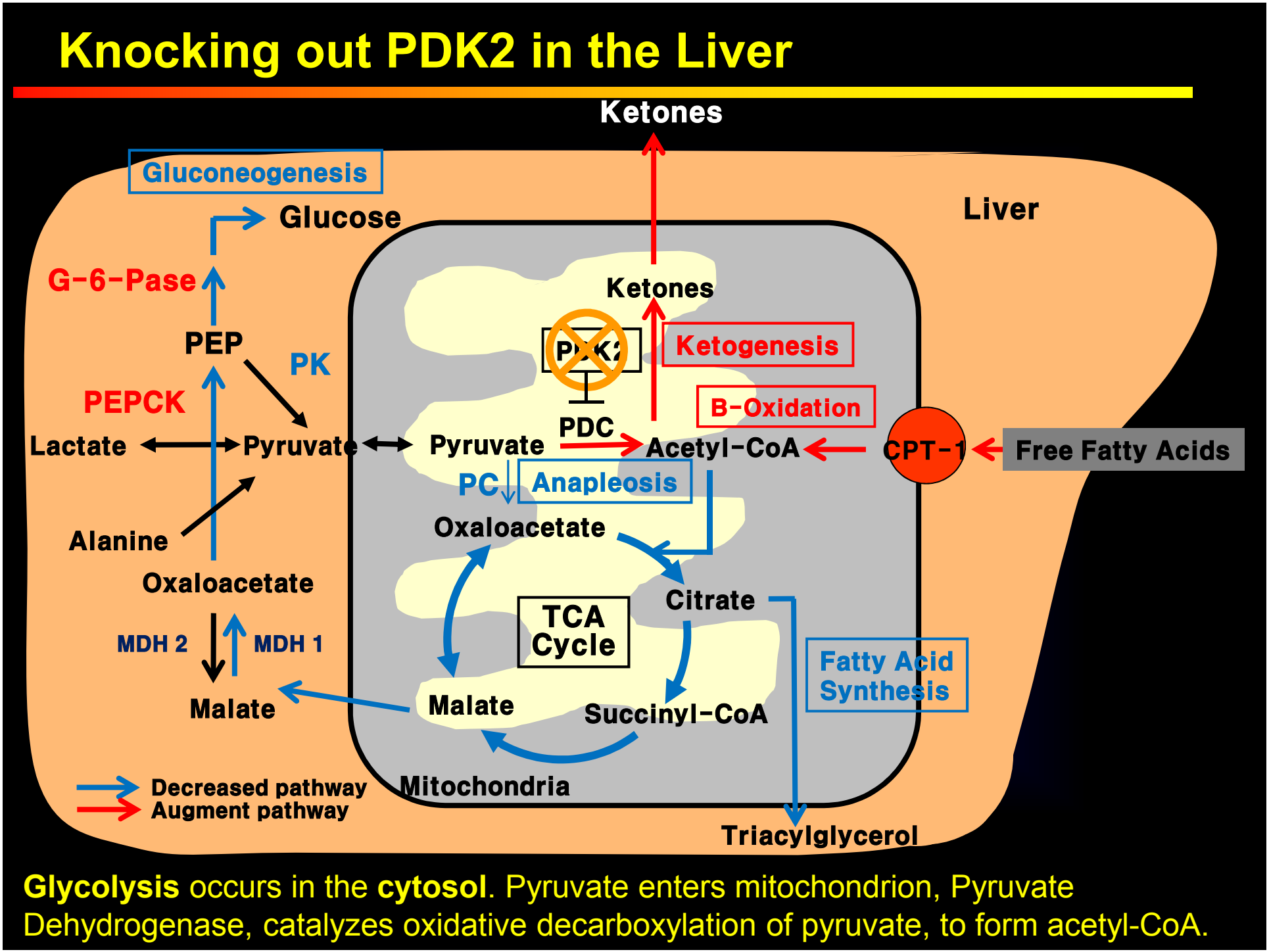
Hepatic insulin signaling pathway in liver of HFD fed WT and PDK2-/- overnight-fasted mice after IP injection of saline and insulin (5U/kg body weight).







Knocking out PDK2 in the Liver



Glycolysis occurs in the **cytosol**. Pyruvate enters mitochondrion, Pyruvate Dehydrogenase, catalyzes oxidative decarboxylation of pyruvate, to form acetyl-CoA.

Summary(2)

- **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 infilteration 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**

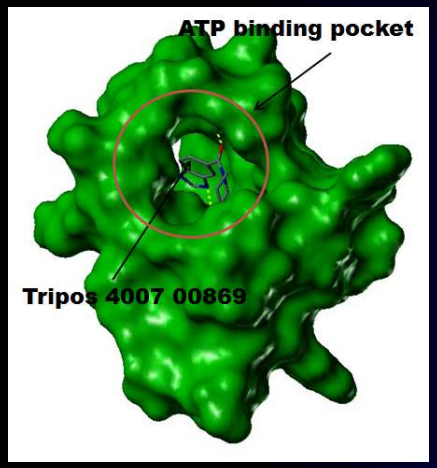
  Ketone bodies will be increased.

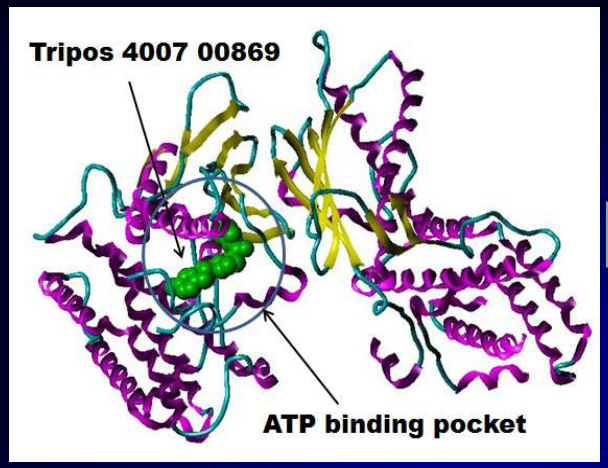
PDK4 Inhibitor

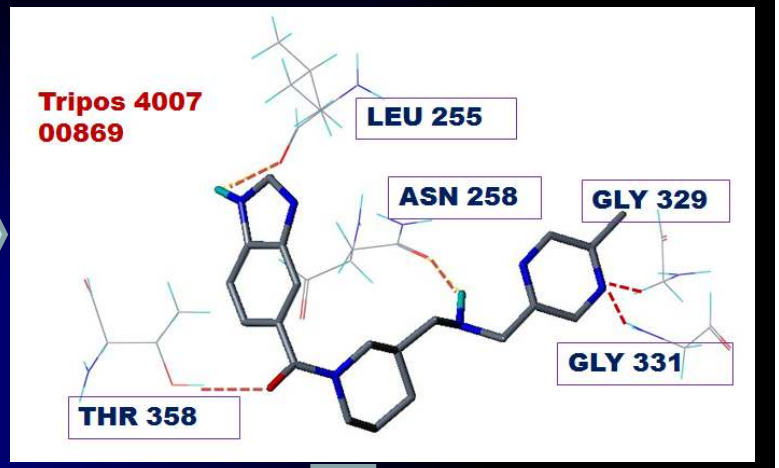
상태	물질	회사	작용기전	적용질환
Phase III	CPC-2011 DCA X-11S	Questcor University of Alberta University of California, San Diego University of Cincinnati	Pyruvate Dehydrogenase Kinase(PHDK; PDK) Inhibitors	Ischemic Stroke, Neurologic disease Solid Tumors and Brain cancer Therapy Disorders of the Coronary Arteries and Atherosclerosis
Preclinical	NSC-294404	Kitasato Institute	Pyruvate Dehydrogenase Kinase(PHDK; PDK) Inhibitors	Anti fungal agents Antineoplastic Antibiotics Antimalaria drugs
Preclinical	279383 279387 279389	Norvatis	Pyruvate Dehydrogenase Kinase(PHDK; PDK) Inhibitors	Anti diabetes drugs
Biologic Testing	283967	Astrazeneca	Pyruvate Dehydrogenase Kinase 2 (PHDK; PDK) Inhibitors	Anti diabetes drugs

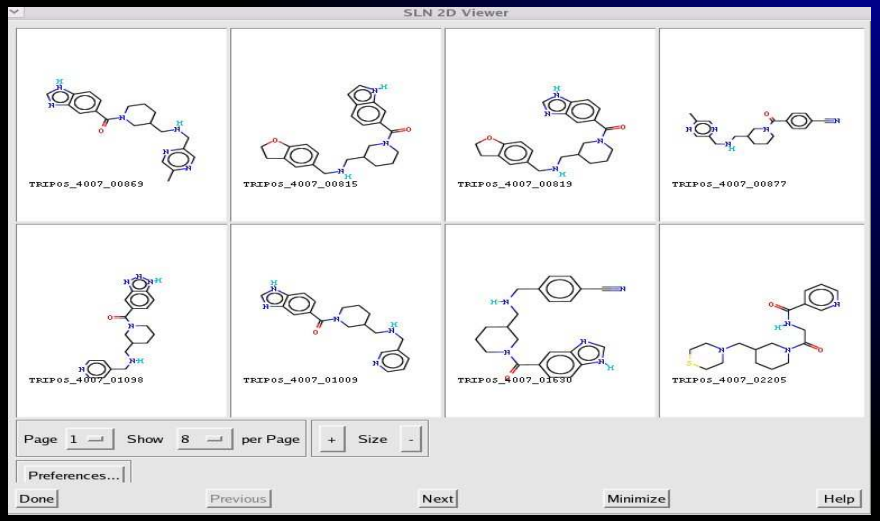
PDK4 Inhibitor

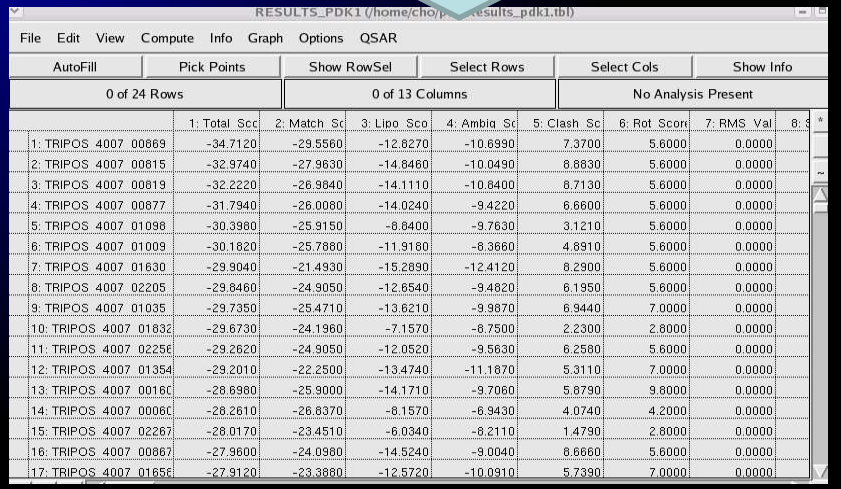
PDK4 inhibitor Synthesis











	1: Total Scc	2: Match Sc	3: Lipo Sco	4: Ambig Sc	5: Clash Sc	6: Rot Scor	7: RMS Val
1: TRIPOS 4007 00869	-34.7120	-29.5560	-12.8270	-10.6990	7.3700	5.6000	0.0000
2: TRIPOS 4007 00815	-32.9740	-27.9630	-14.8460	-10.0490	8.8830	5.6000	0.0000
3: TRIPOS 4007 00819	-32.2220	-26.9840	-14.1110	-10.8400	8.7130	5.6000	0.0000
4: TRIPOS 4007 00877	-31.7940	-26.0080	-14.0240	-9.4220	6.6600	5.6000	0.0000
5: TRIPOS 4007 01098	-30.3980	-25.9150	-8.6400	-9.7630	3.1210	5.6000	0.0000
6: TRIPOS 4007 01009	-30.1820	-25.7880	-11.9180	-8.3660	4.8910	5.6000	0.0000
7: TRIPOS 4007 01630	-29.9040	-21.4930	-15.2890	-12.4120	8.2900	5.6000	0.0000
8: TRIPOS 4007 02205	-29.8460	-24.9050	-12.6540	-9.4820	6.1950	5.6000	0.0000
9: TRIPOS 4007 01035	-29.7350	-25.4710	-13.6210	-9.9870	6.9440	7.0000	0.0000
10: TRIPOS 4007 01832	-29.6730	-24.1960	-7.1570	-8.7500	2.2300	2.8000	0.0000
11: TRIPOS 4007 0225(	-29.2620	-24.9050	-12.0520	-9.5630	6.2580	5.6000	0.0000
12: TRIPOS 4007 01354	-29.2010	-22.2500	-13.4740	-11.1870	5.3110	7.0000	0.0000
13: TRIPOS 4007 0016(	-28.6980	-25.9000	-14.1710	-9.7060	5.8790	9.8000	0.0000
14: TRIPOS 4007 0006(	-28.2610	-26.8370	-8.1570	-6.9430	4.0740	4.2000	0.0000
15: TRIPOS 4007 02267	-28.0170	-23.4510	-6.0340	-8.2110	1.4790	2.8000	0.0000
16: TRIPOS 4007 00867	-27.9600	-24.0980	-14.5240	-9.0040	8.6660	5.6000	0.0000
17: TRIPOS 4007 0165(	-27.9120	-23.3880	-12.5720	-10.0910	5.7390	7.0000	0.0000

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