International Conference on Diabetes and Metabolism

Tissue Specific Roles of PDK2 and 4 in Regulation of Blood Glucose Levels

Robert A. Harris
Roudebush VA Medical Center
Biochemistry and Molecular Biology
Indiana University School of Medicine

Kyungpook National University, Daegu

Professor In-kyu Lee
Younghoon Go
Ji Yun Jeong
Nam Ho Jeoung

Indiana University, Indianapolis

Yasmeen Rahimi
Pengfei Wu

PDC is active in the FED state, inactive in the FASTED state

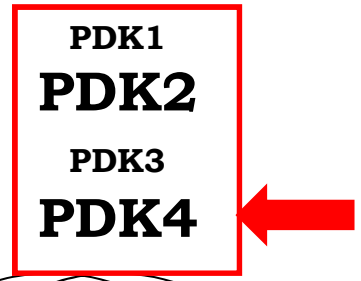
FED STATE

Pyruvate + CoASH + NAD

Acetyl-CoA + CO_2 + NADH

Good metabolic flexibility

FASTED STATE



Induced

Pyruvate + CoASH + NAD

Acetyl-CoA + CO_2 + NADH

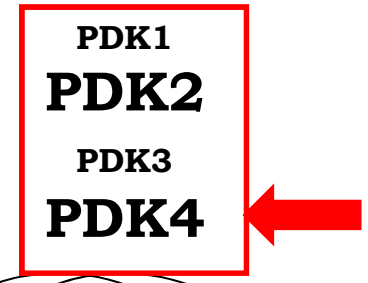
PDC is inactive in DIABETES

Non diabetic fed state

Pyruvate + CoASH + NAD

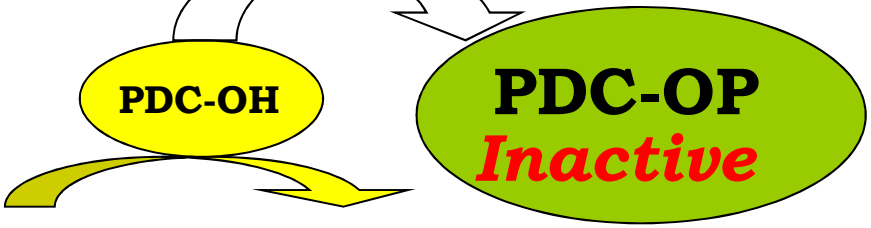
Acetyl-CoA + CO_2 + NADH

Diabetes fed and fasted states



Induced

Poor metabolic flexibility



Pyruvate + CoASH + NAD

Acetyl-CoA + CO_2 + NADH

PDK KO mice have been made to test whether PDKs are druggable targets

PDK2 KO mice

PDK4 KO mice

PDK2/PDK4 DKO mice

Viable; normal growth and appearance; no evidence of neurologic dysfunction

PDK deficiency lowers blood glucose levels

Genotype	Blood Glucose	
	Fed	Fasted
	mg/dl	
WT	174 ± 6	94 ± 7
PDK2 KO	149 ± 3*	87 ± 7
PDK4 KO	177 ± 11	75 ± 2*
PDK2/PDK4 DKO	154 ± 4*	61 ± 2* #

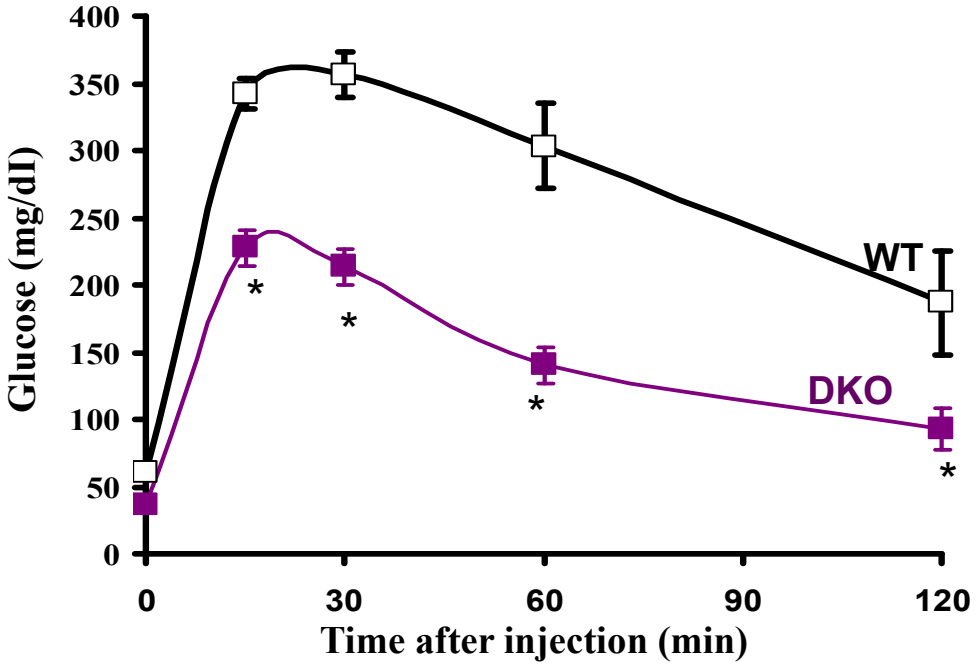
PDK2 deficiency increases liver PDC activity state

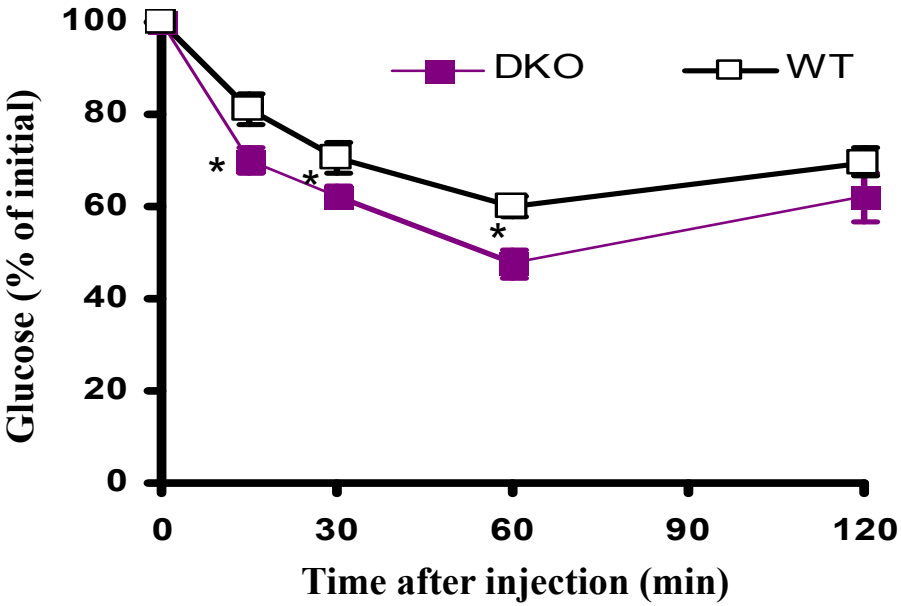
		PDC		
Tissue	Genotype	Actual activity	Total activity	Activity State
		μmol/min/g wet weight tissue		% active
Liver	WT	0.7 ± 0.1	2.47 ± 0.08	30 ± 4
	PDK2 KO	1.3 ± 0.1	2.54 ± 0.08	51 ± 4*
	PDK4 KO	0.8 ± 0.2	2.3 ± 0.1	32 ± 8
	PDK2/PDK4 DKO	1.2 ± 0.1	2.28 ± 0.06	56 ± 5*

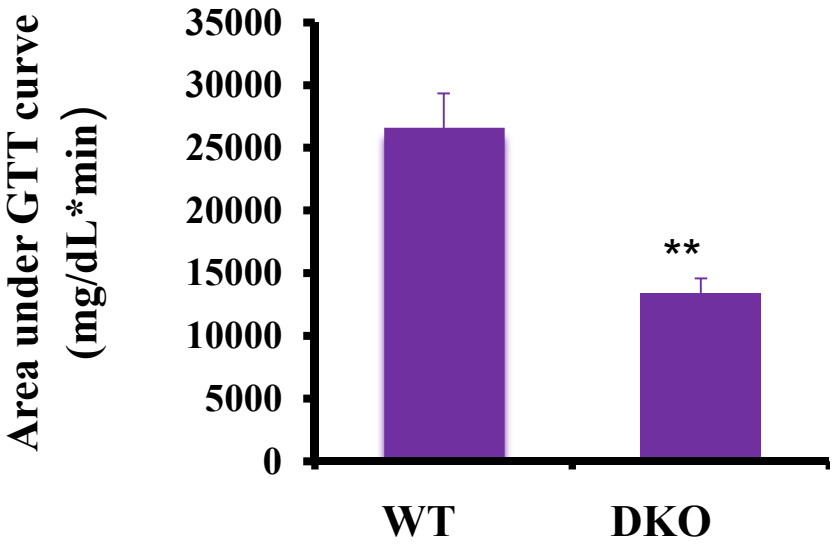
PDK4 deficiency increases muscle PDC activity state

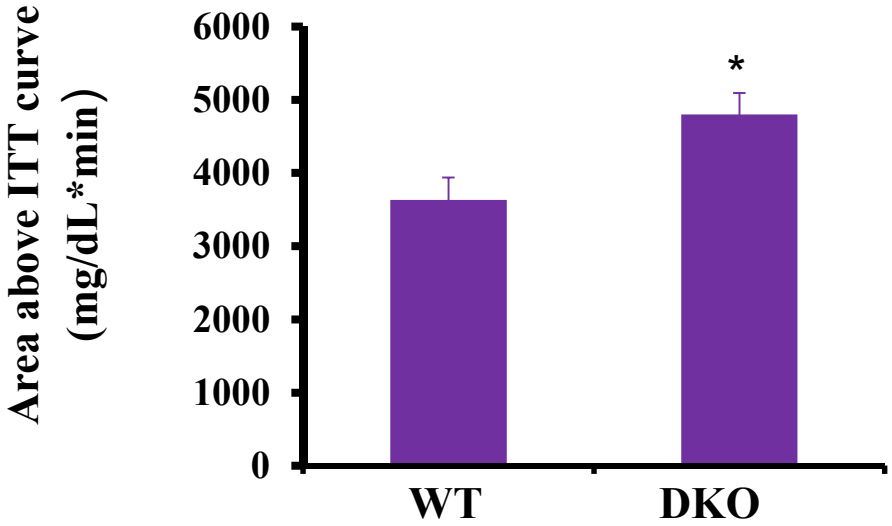
		PDC		
Tissue	Genotype	Actual activity	Total activity	Activity State
		μmol/min/g wet weight tissue		% active
Muscle	WT	0.36 ± 0.01	2.56 ± 0.03	14.0 ± 0.4
	PDK2 KO	0.34 ± 0.02	2.56 ± 0.02	14.3 ± 0.8
	PDK4 KO	1.0 ± 0.1*	2.69 ± 0.02	39 ± 4*
	PDK2/PDK4 DKO	1.85 ± 0.03*	2.73 ± 0.02	67 ± 1*

PDK deficiency improves glucose and insulin tolerance



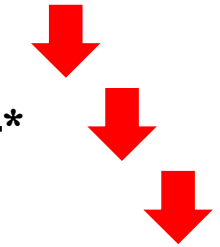






PDK deficiency reduces blood levels of gluconeogenic substrates

Measurement	Units	WT	DKO
Lactate	mmol/L	2.64 ± 0.14	1.49 ± 0.01*
Pyruvate	mmol/L	0.107 ± 0.013	0.029 ± 0.004*
Alanine	mmol/L	0.21 ± 0.02	0.11 ± 0.01*



Reduced substrate supply limits hepatic gluconeogenesis, resulting in low blood glucose levels in PDK deficient mice.

PDK deficiency prevents hyperglycemia in mice fed a high fat diet by lowering gluconeogenic substrates

Measurement		WT	DKO
Glucose	mg/dL	192 ± 11	107 ± 4* ↓
Lactate	mmol/L	2.2 ± 0.3	0.9 ± 0.3* ↓
Pyruvate	mmol/L	0.13 ± 0.02	0.017 ± 0.004* ↓
Alanine	mmol/L	0.26 ± 0.04	0.11 ± 0.03* ↓

Determination of glucose production rates by stable isotope metabolic flux studies

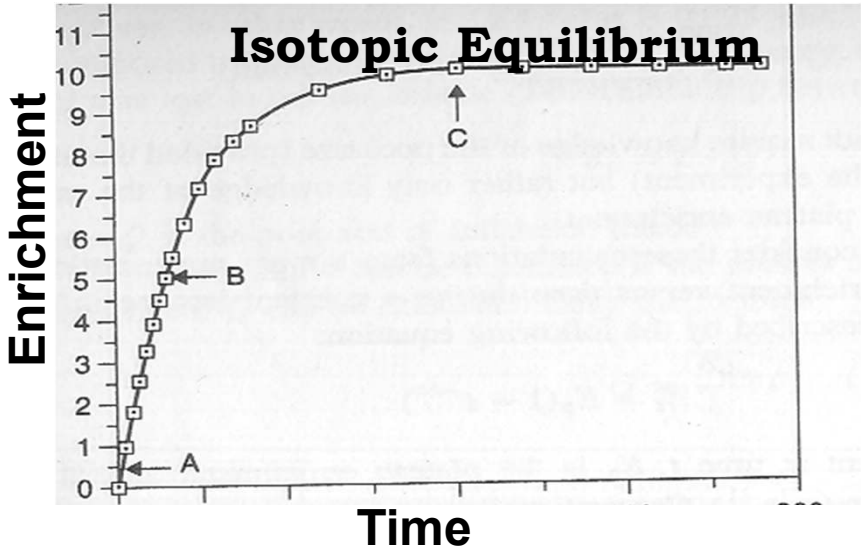
[U-$^{13}C_6$] glucose

Collect blood

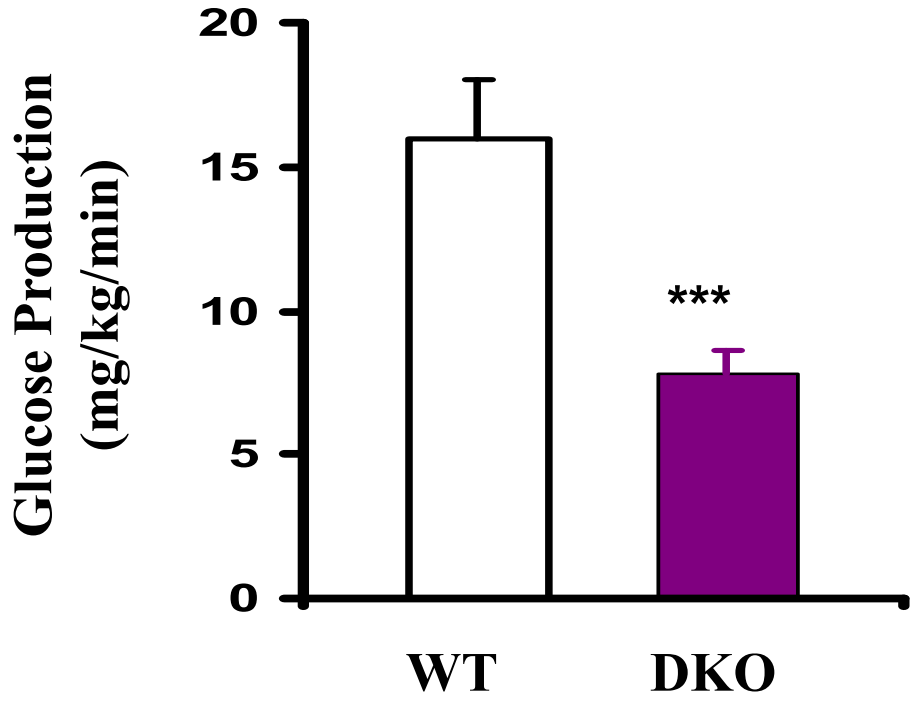
Analysis Enrichment by GC/MS

Determine enrichment at isotopic equilibrium (Ep)

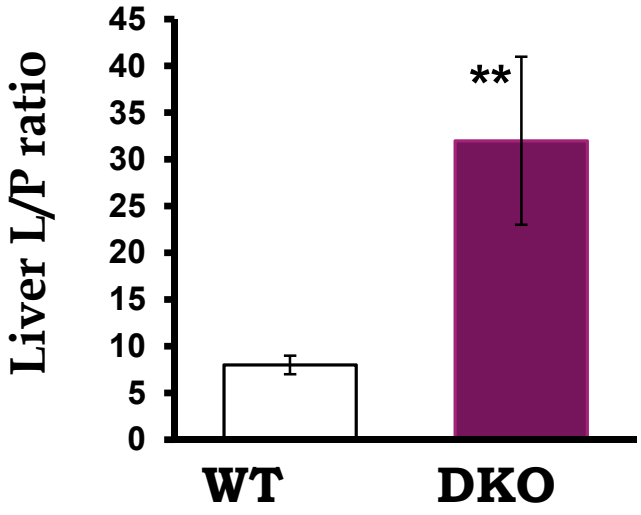
Calculate rate of glucose production



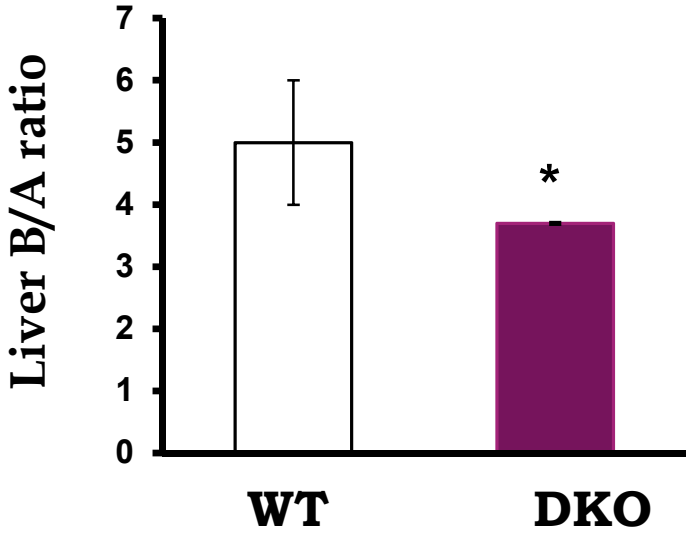
PDK deficiency reduces the rate of glucose production



PDK deficiency induces opposite effects on the NADH/NAD ratio in the cytosol and the mitochondrial matrix space



Increased cytosolic NADH/NAD ratio



Decreased mitochondrial NADH/NAD ratio

PDK deficiency induces phenotype similar to pyruvate carboxylase deficiency

Pyruvate carboxylase deficiency causes high lactate/pyruvate ratio (high cytosolic NADH/NAD ratio) and low beta-hyroxybutyrate/acetoacetate ratio (low mitochondrial NADH/NAD ratio).

PDK deficiency reduces substrate supply for pyruvate carboxylase activity, resulting in a phenotype similar to pyruvate carboxylase deficiency.

PDK deficiency reduces liver levels of pyruvate and citric acid cycle intermediates

Measurement	WT	DKO
	μmol/gram wet weight	
Pyruvate	0.019 ± 0.003	0.009 ± 0.002*
OAA	0.015 ± 0.002	0.009 ± 0.002*
OAA calculated	0.012 ± 0.002	0.004 ± 0.003*
Citrate	0.26 ± 0.04	0.13 ± 0.02*
α-Ketoglutarate	0.016 ± 0.001	0.011 ± 0.001*
ATP	2.4 ± 0.1	2.25 ± 0.07

PDKs conserves pyruvate for the production of citric acid cycle intermediates by anaplerosis.

Mechanism responsible for reduced glucose synthesis

- Increased pyruvate oxidation and cytoplasmic NADH/NAD ratio result in a low [pyruvate] that limits pyruvate carboxylase activity:

  Pyruvate + CO_2 + ATP $\rightarrow$ OAA + ADP + Pi

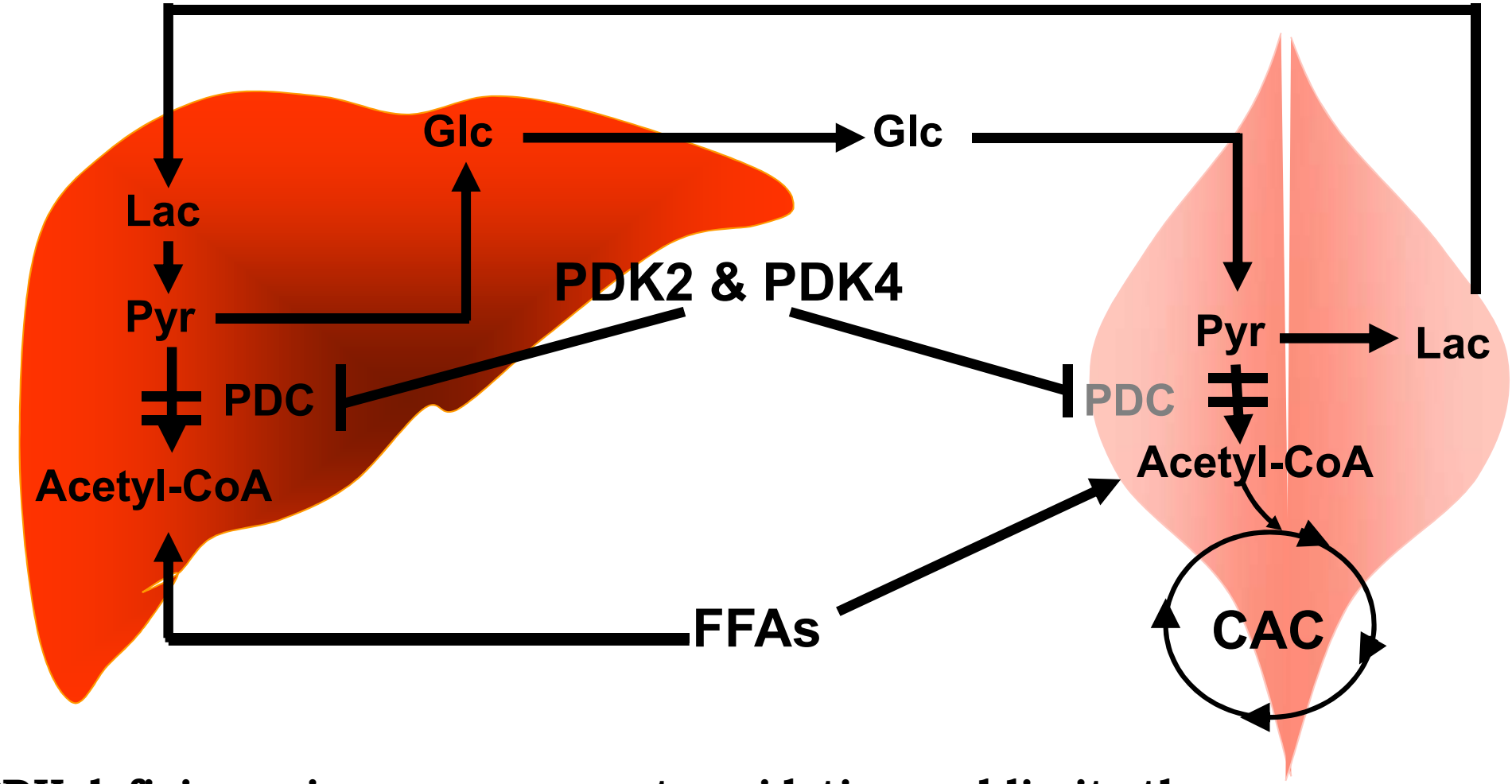
- Decreased formation of OAA and high cytoplasmic NADH/NAD ratio cause a low cytosolic [OAA]:

  OAA + NADH $\rightarrow$ malate + NAD

- The low cytosolic [OAA] limits PEPCK activity:

  OAA + GTP $\rightarrow$ PEP + GDP + CO_2

Why are blood glucose levels reduced in PDK KO mice?



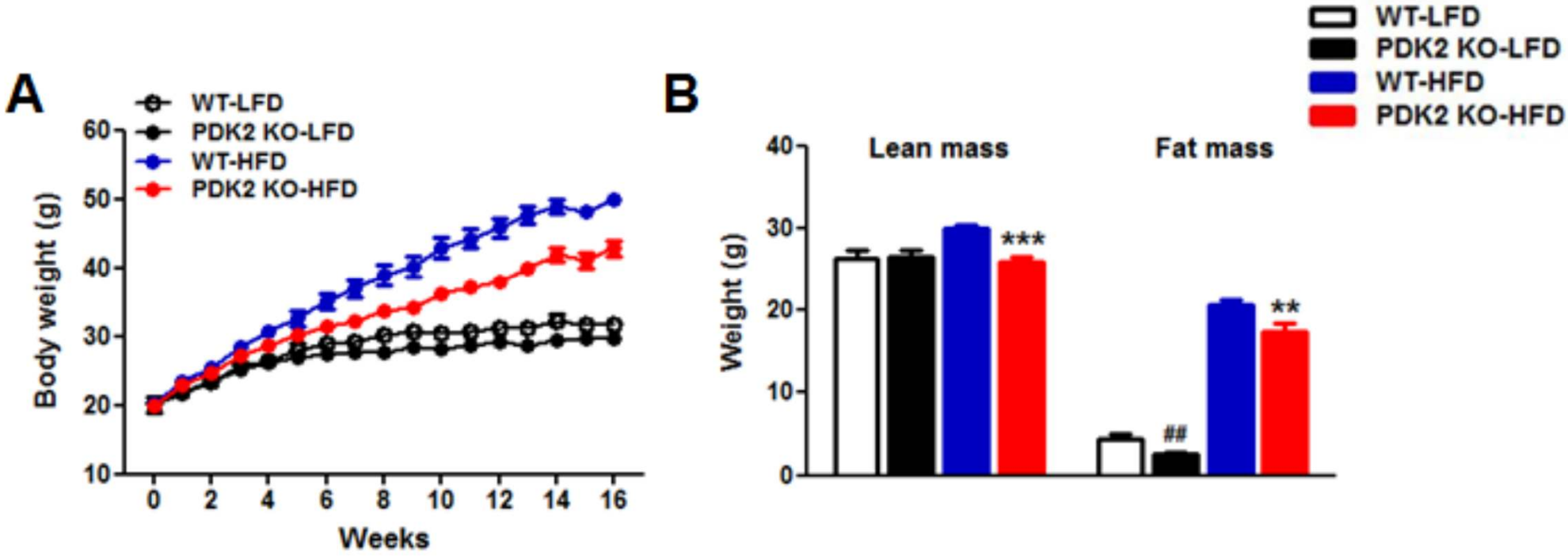
PDK deficiency increases pyruvate oxidation and limits the availability of substrates (pyruvate, lactate, alanine) that can be used for gluconeogenesis.

Based on our findings with PDK4 KO and PDK2/PDK4 DKO mice, we predicted PDK2 deficiency would have little effect on glucose homeostasis.

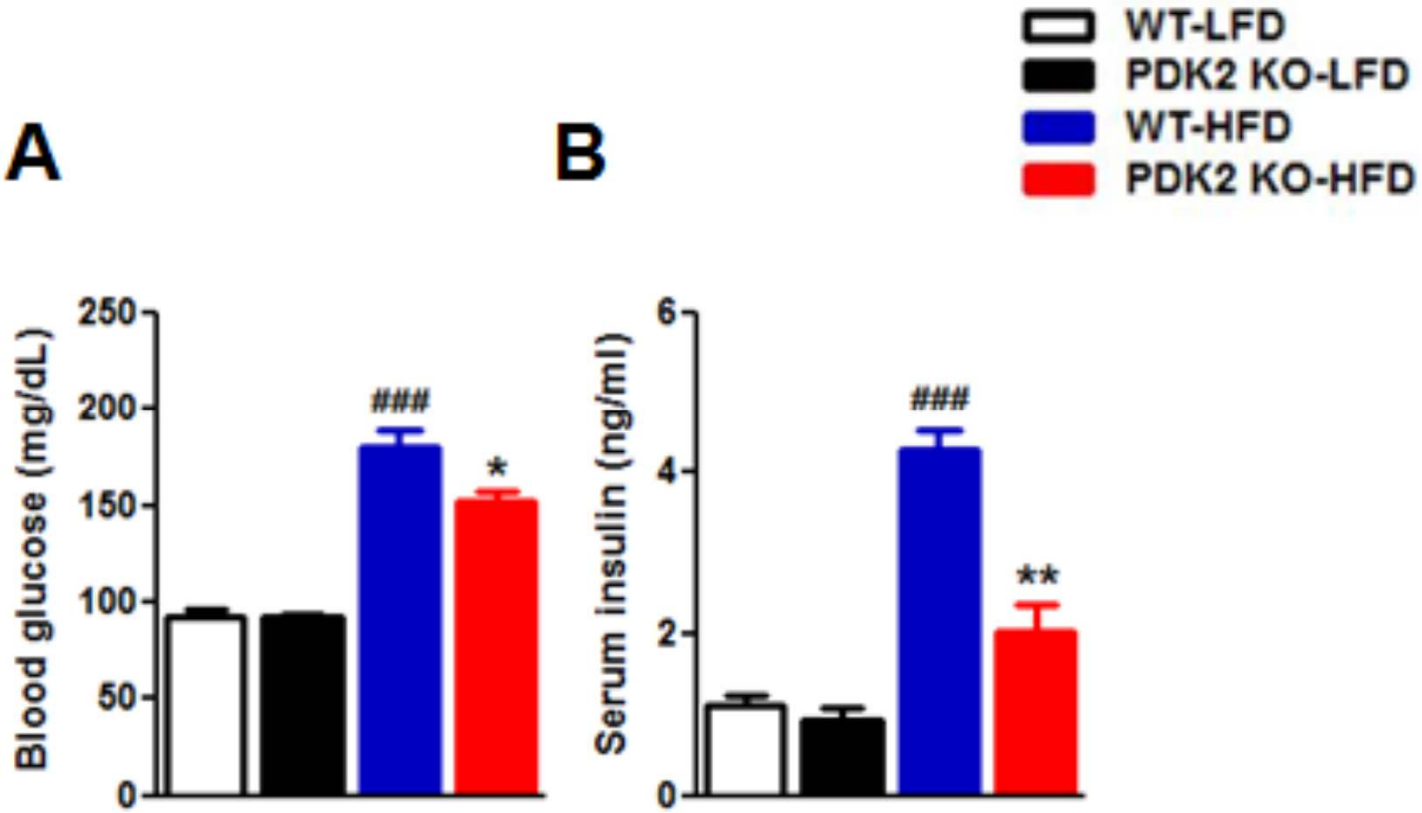
Based on our findings with PDK4 KO and PDK2/PDK4 DKO mice, we predicted PDK2 deficiency would have little effect on glucose homeostasis.

We were wrong……

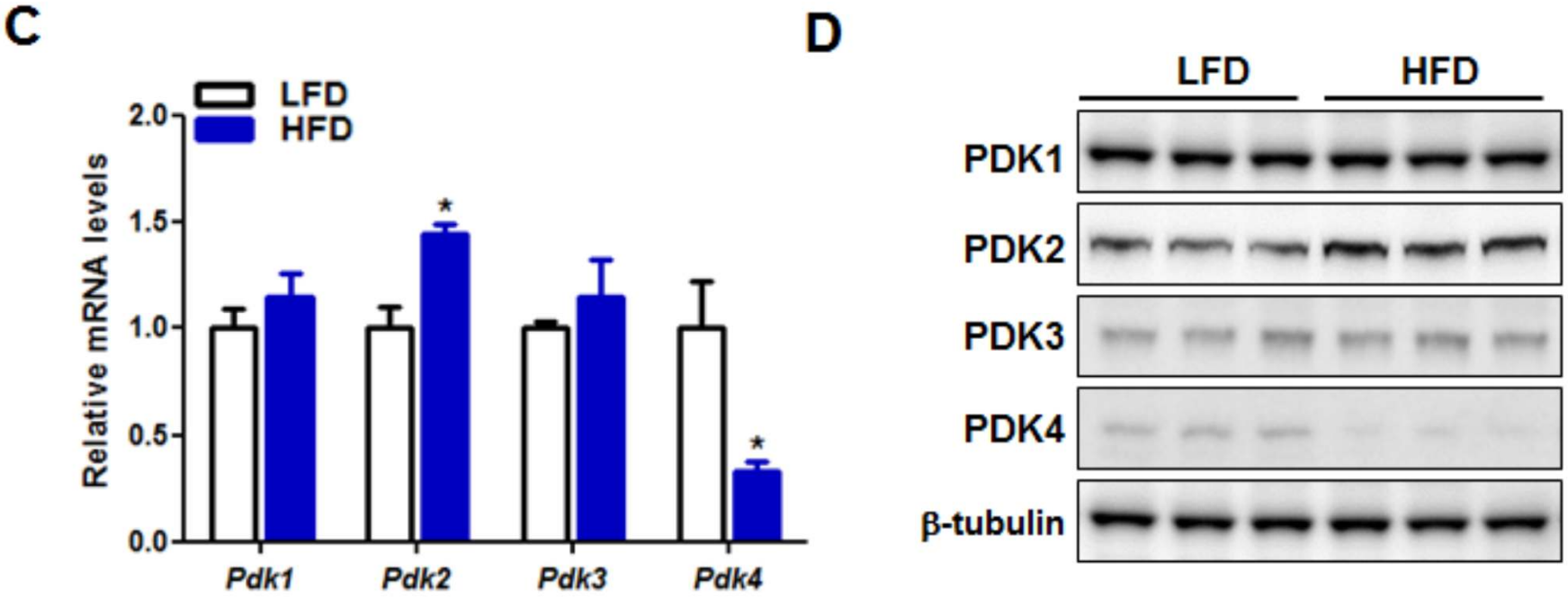
PDK2 deficiency reduces fat mass in LFD- and HFD-fed mice



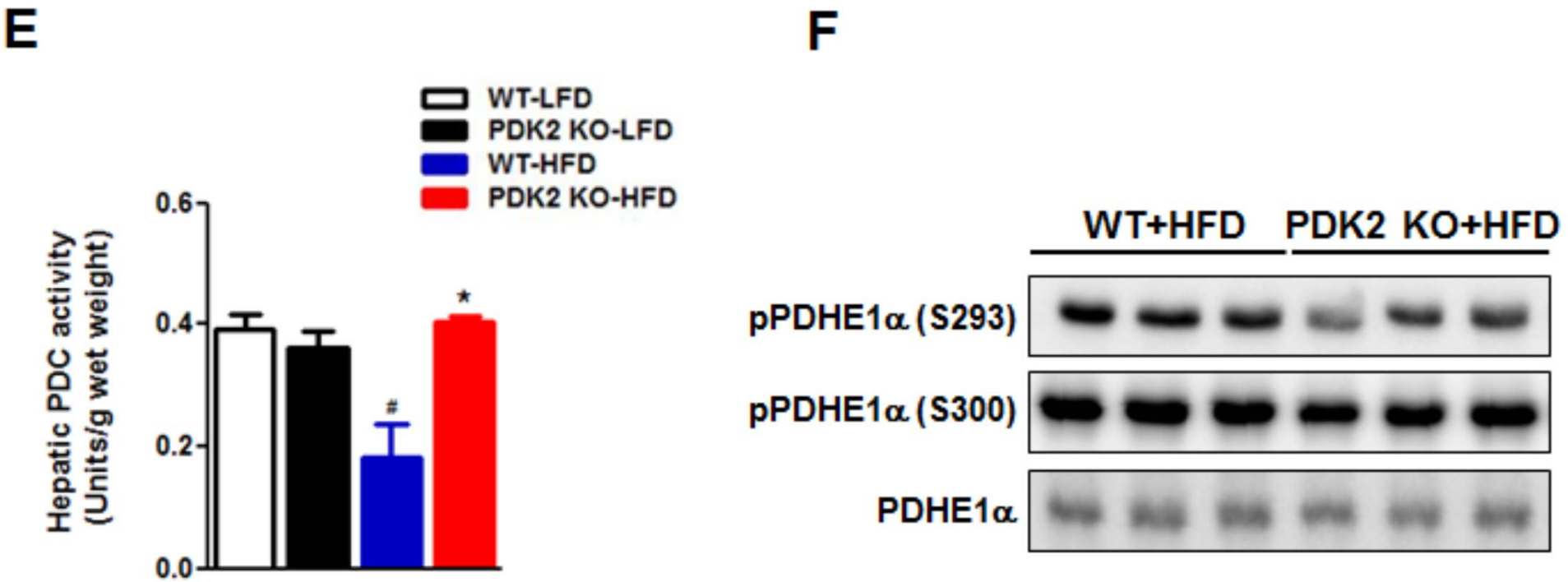
PDK2 deficiency reduces blood glucose and serum insulin in HFD fed mice



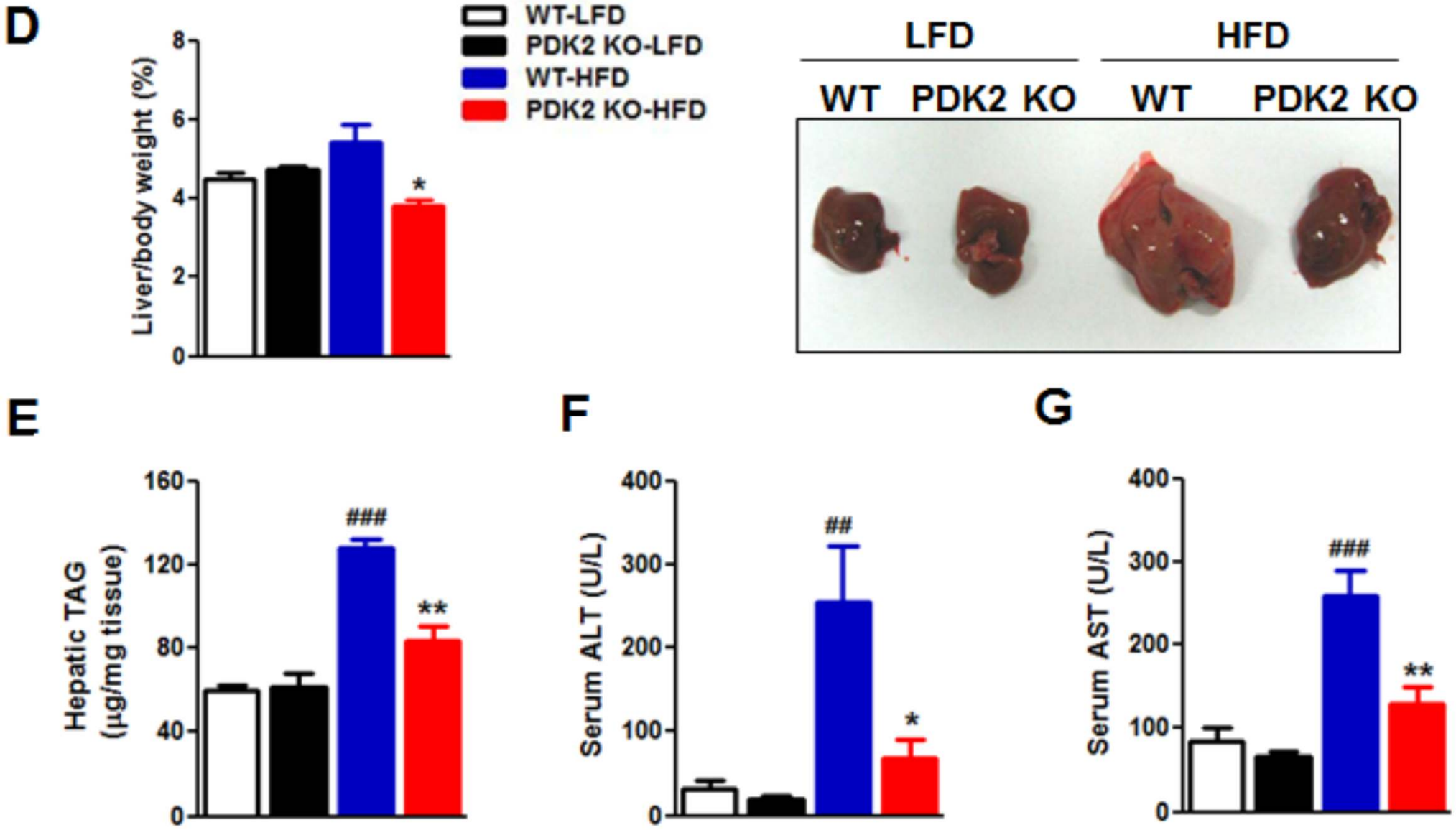
High fat diet increases PDK2 expression but decreases PDK4 expression in the liver



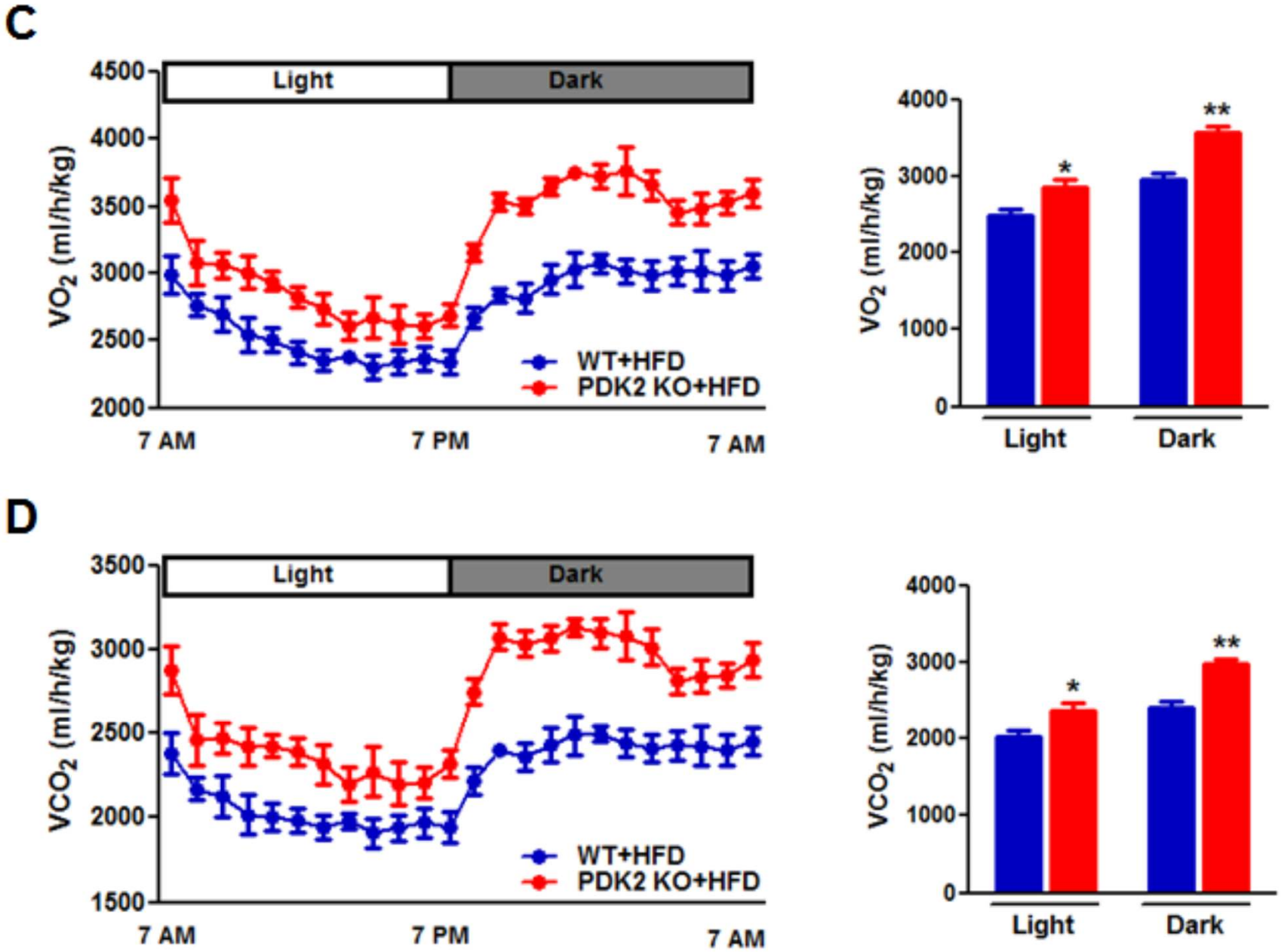
PDK2 deficiency increases PDC activity in the liver of mice fed high fat diet



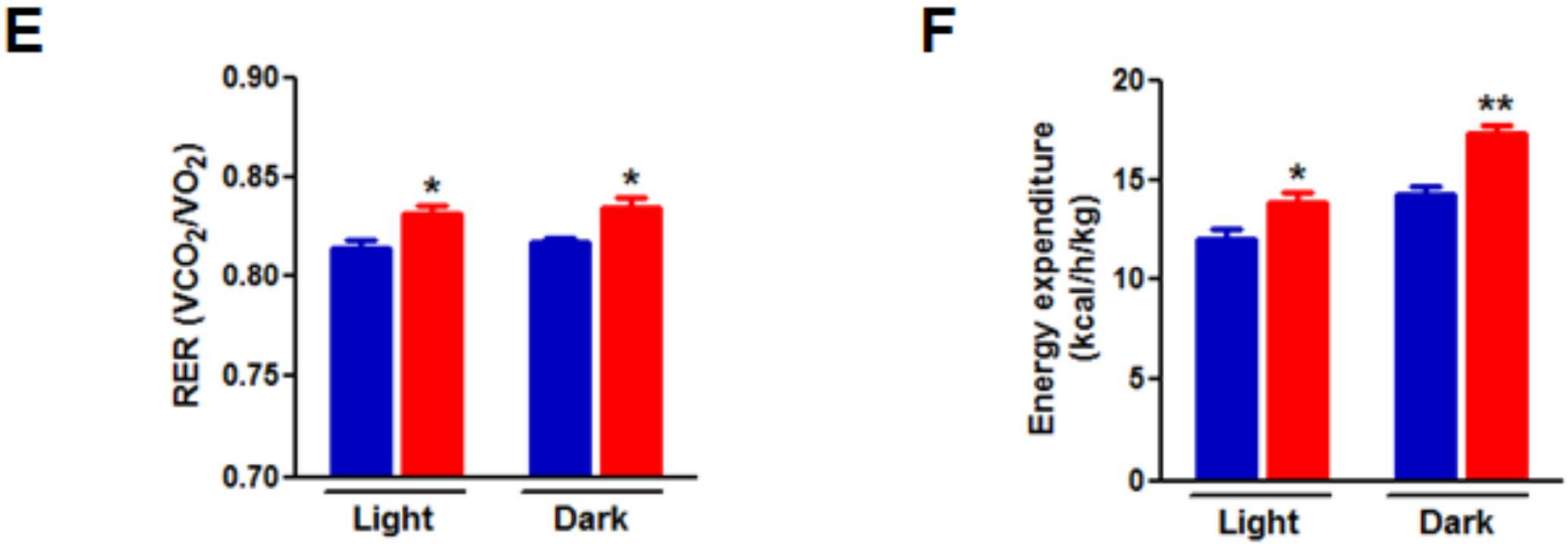
PDK2 deficiency reduces liver weight, liver TAG, and serum ALT and AST



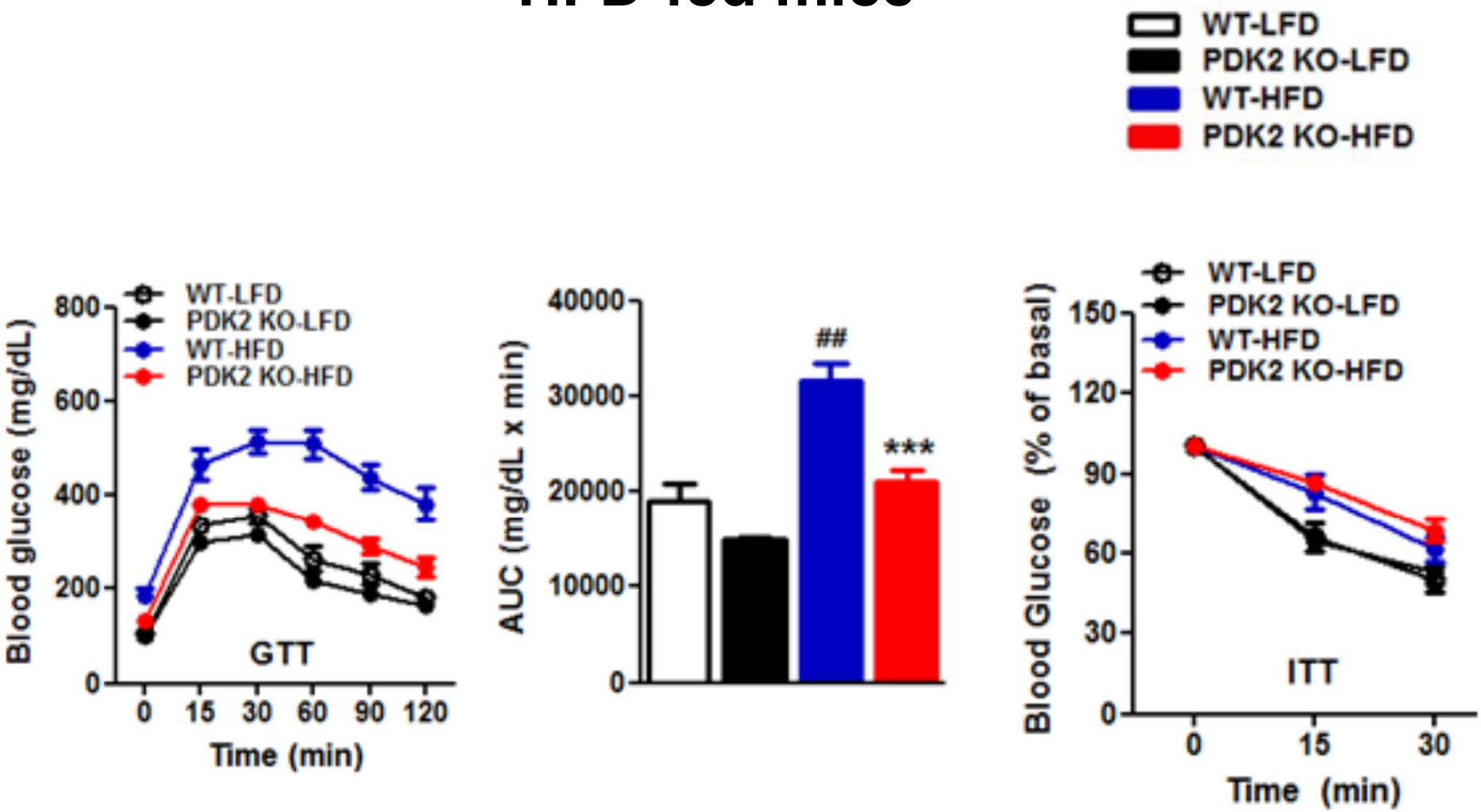
PDK2 deficiency increases both VO_2 and VCO_2 in HFD-fed mice



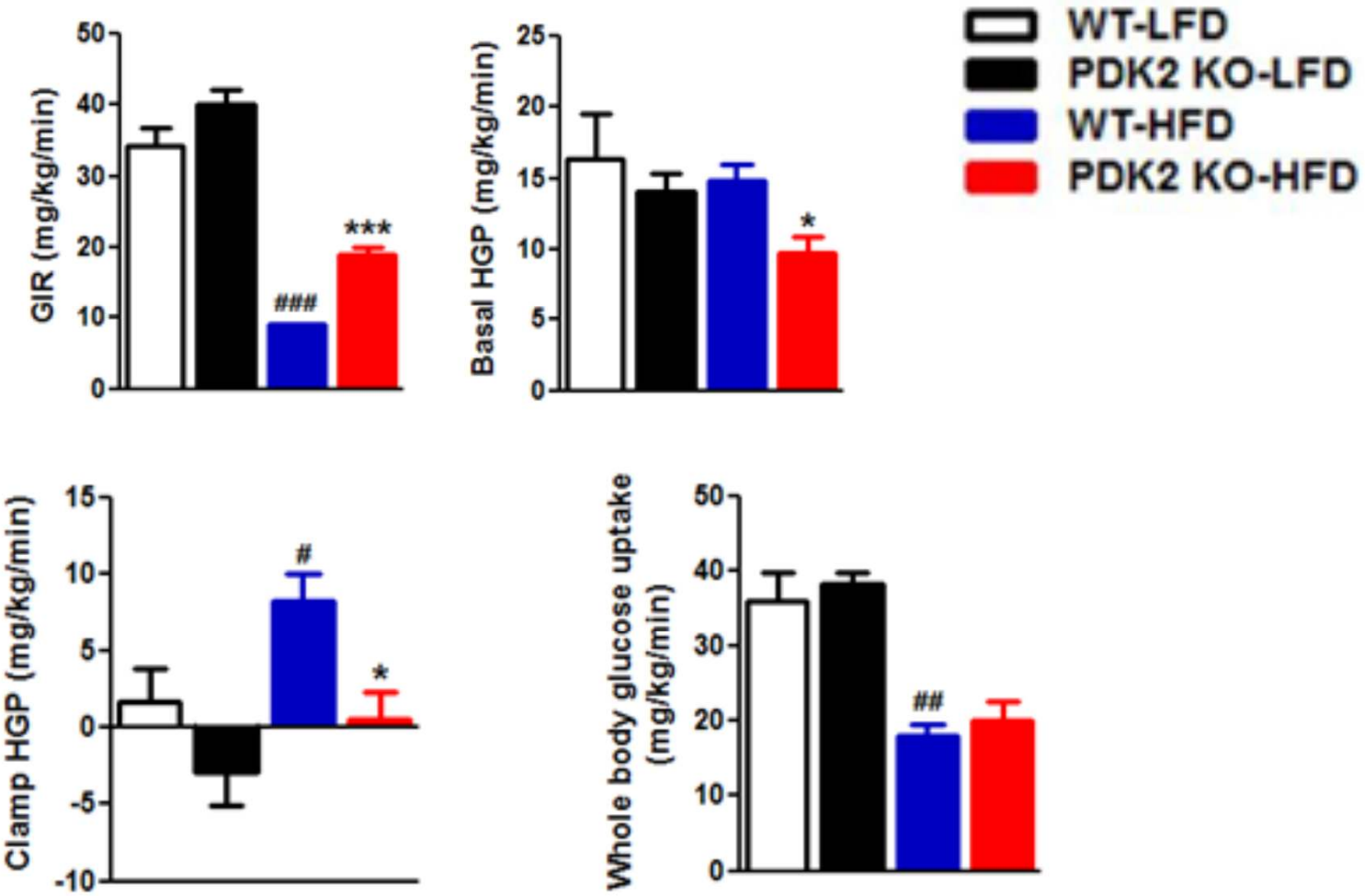
PDK2 deficiency increases RER and energy expenditure in HFD fed mice



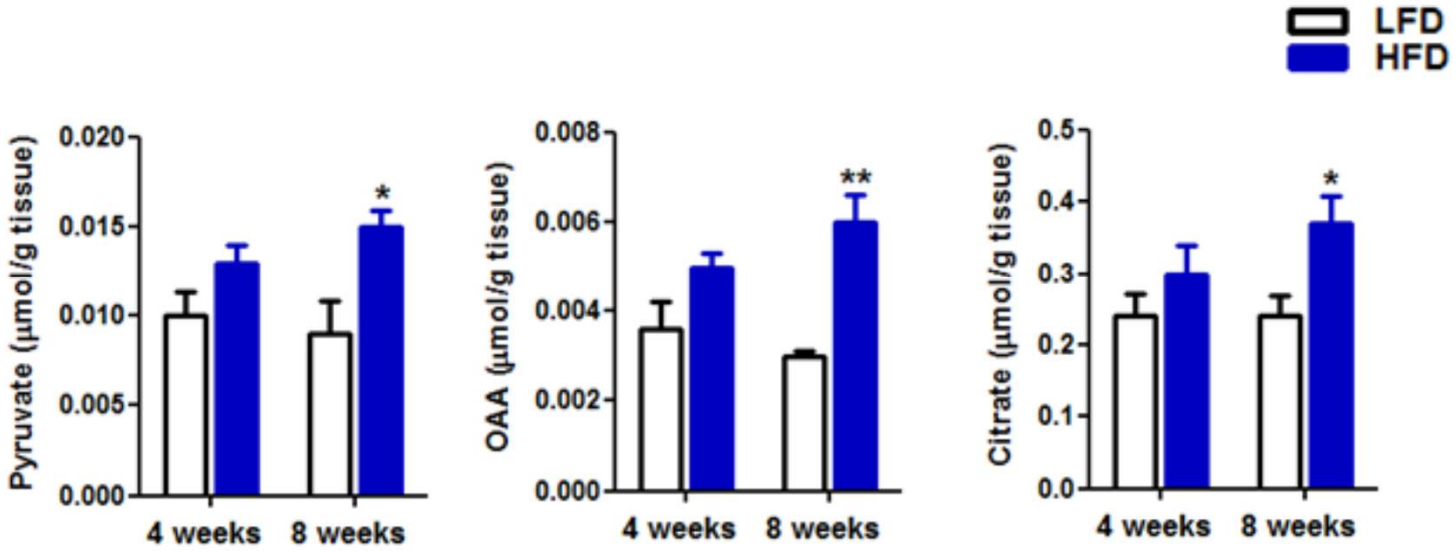
PDK2 deficiency improves GTT but not ITT in HFD-fed mice



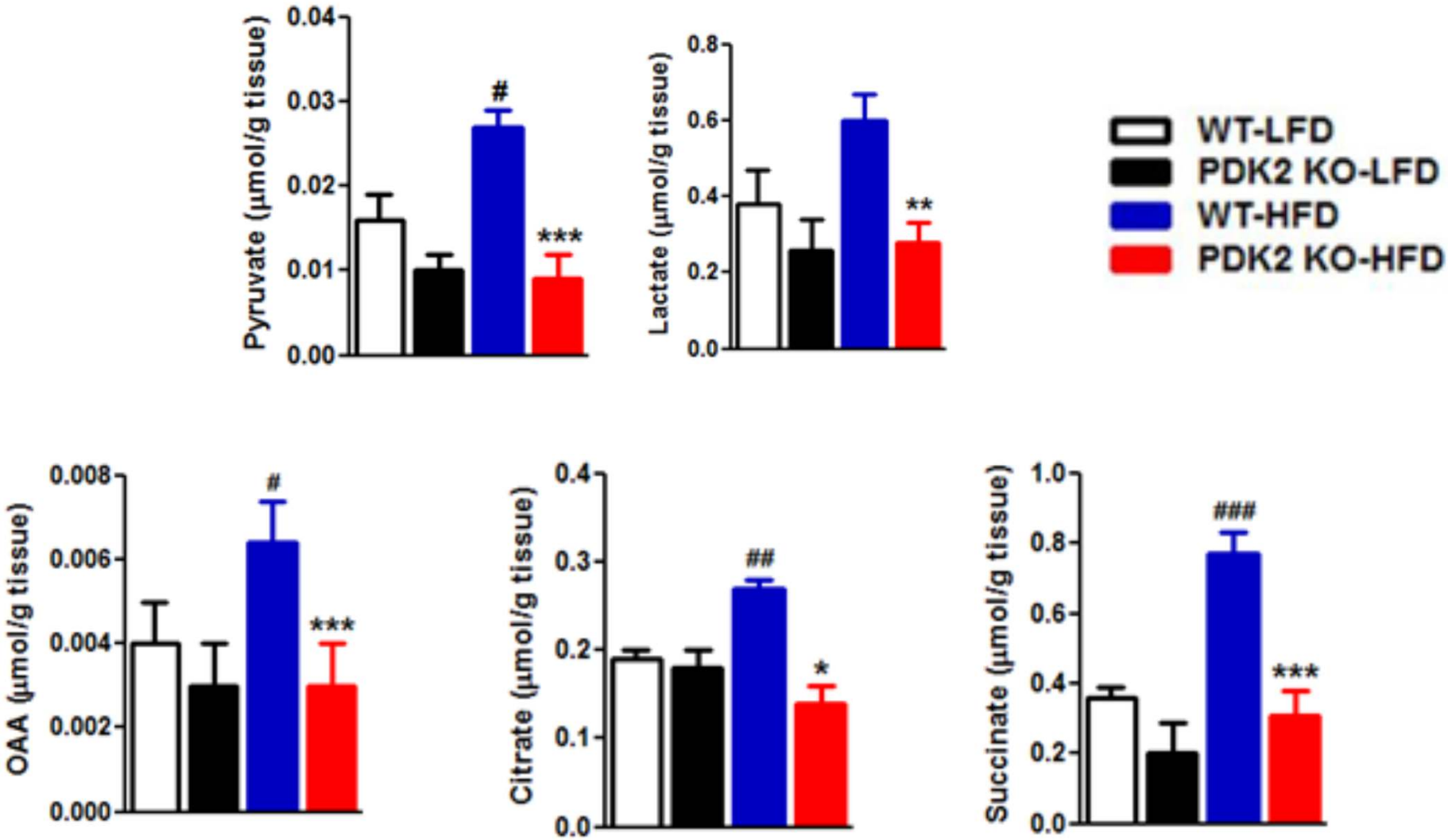
Hyperinsulinemic-euglycemic clamp study
PDK2 deficiency reduces hepatic glucose production in HFD-fed mice



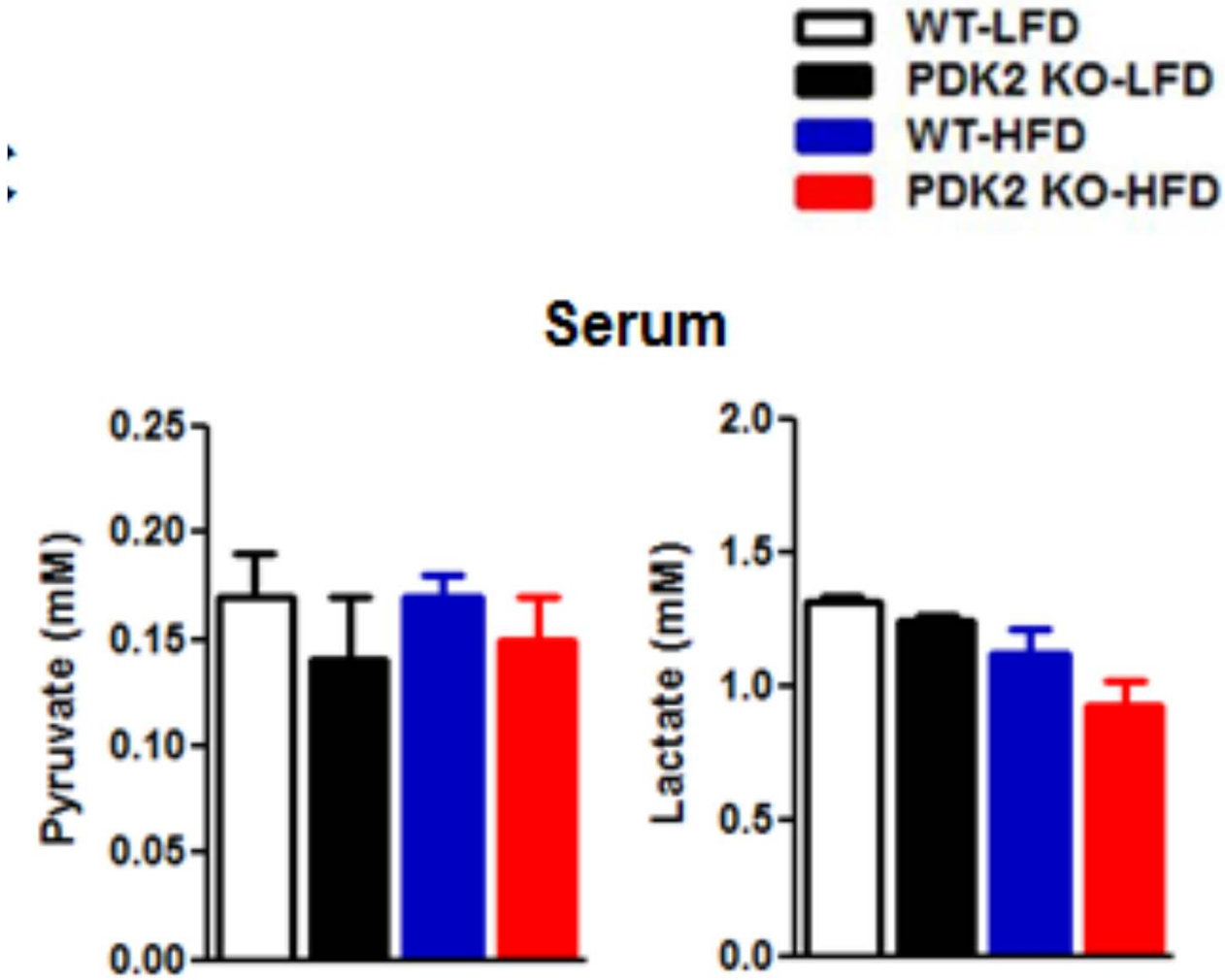
Pyruvate, oxaloacetate, and citrate are increased in the liver in HFD-fed mice



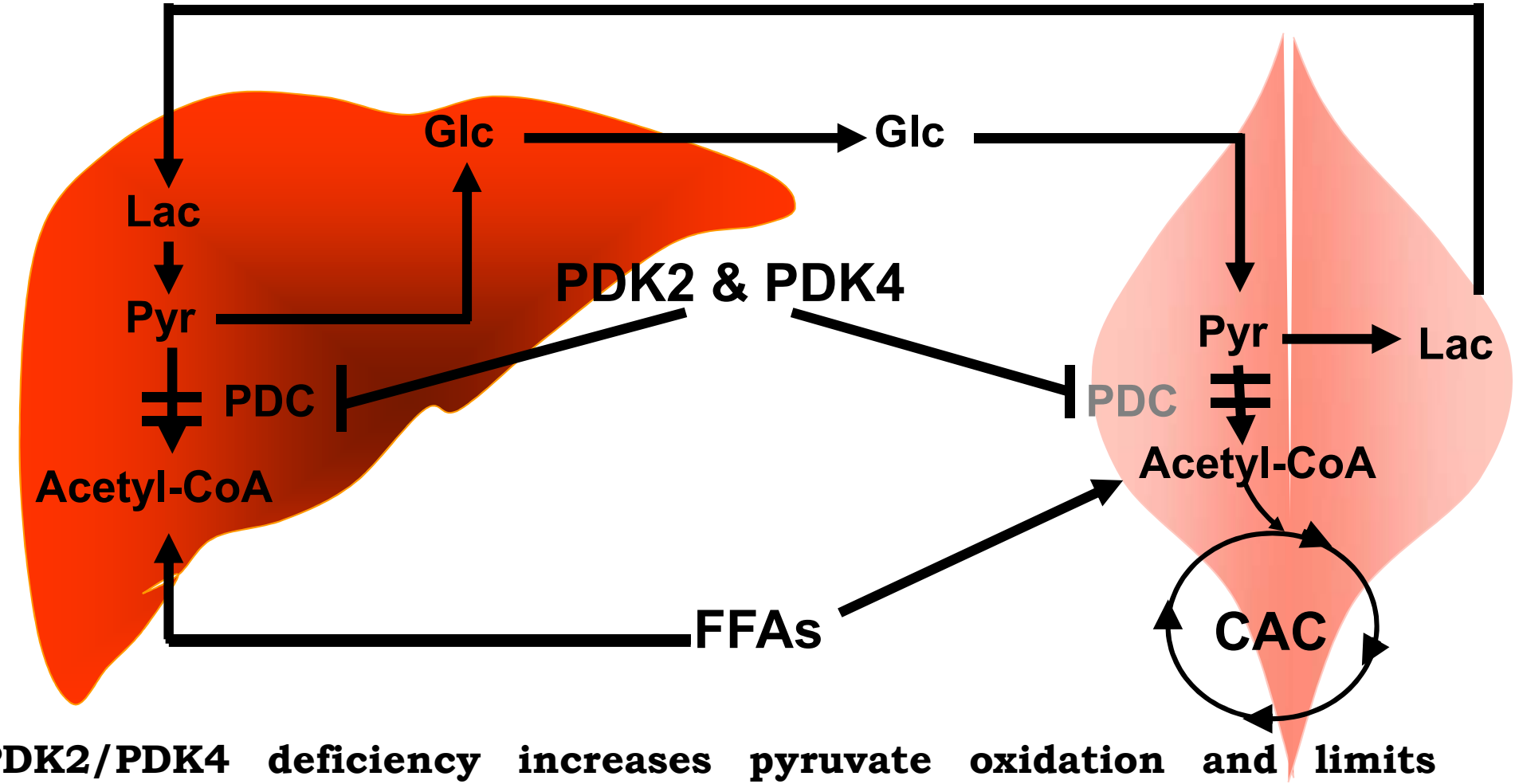
PDK2 deficiency lowers pyruvate, lactate, OAA, citrate, and succinate in the liver of HFD-fed mice



PDK2 deficiency does not decrease pyruvate and lactate in the serum of HFD-fed mice

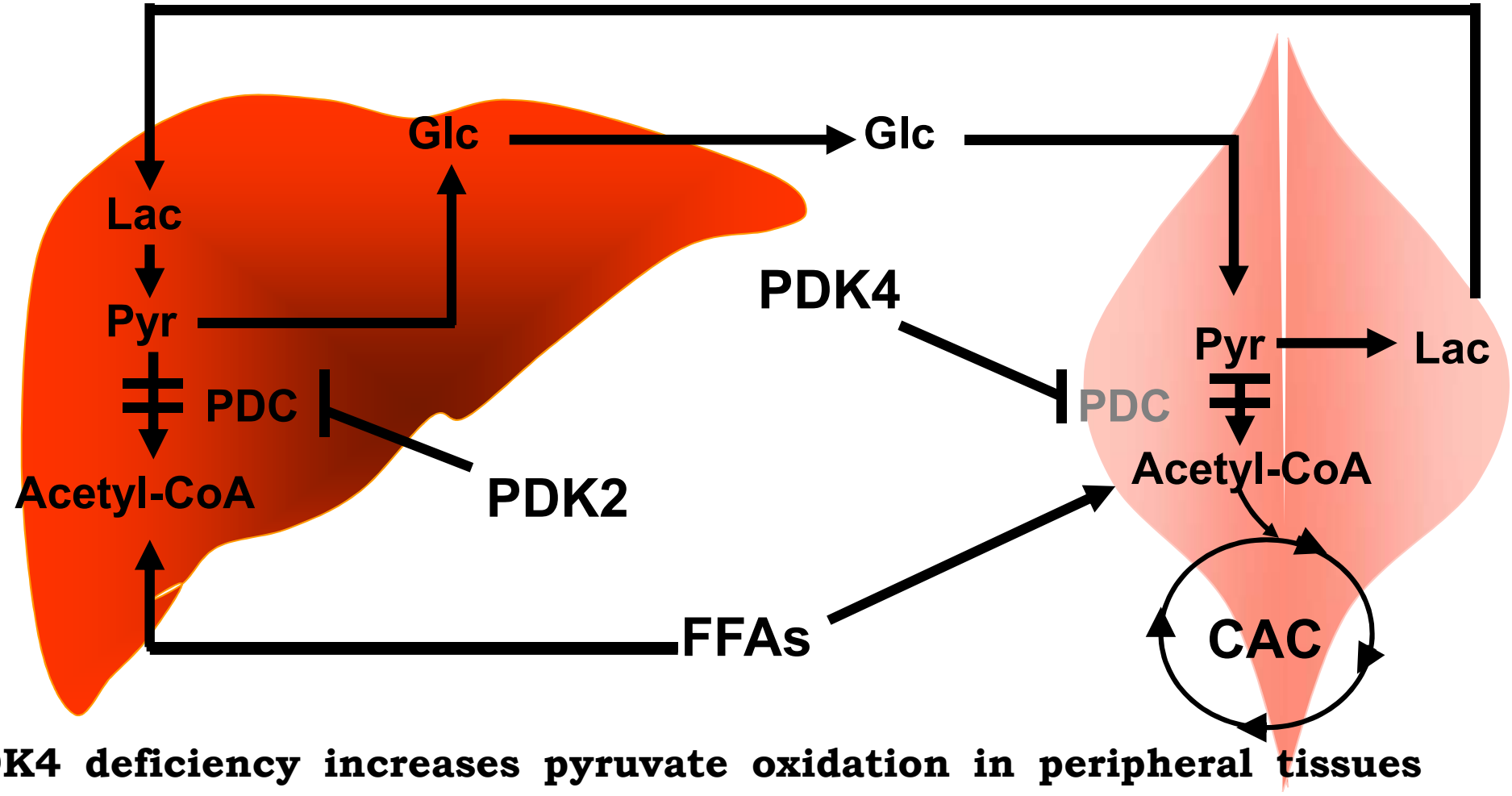


Why are blood glucose levels reduced in PDK KO mice?



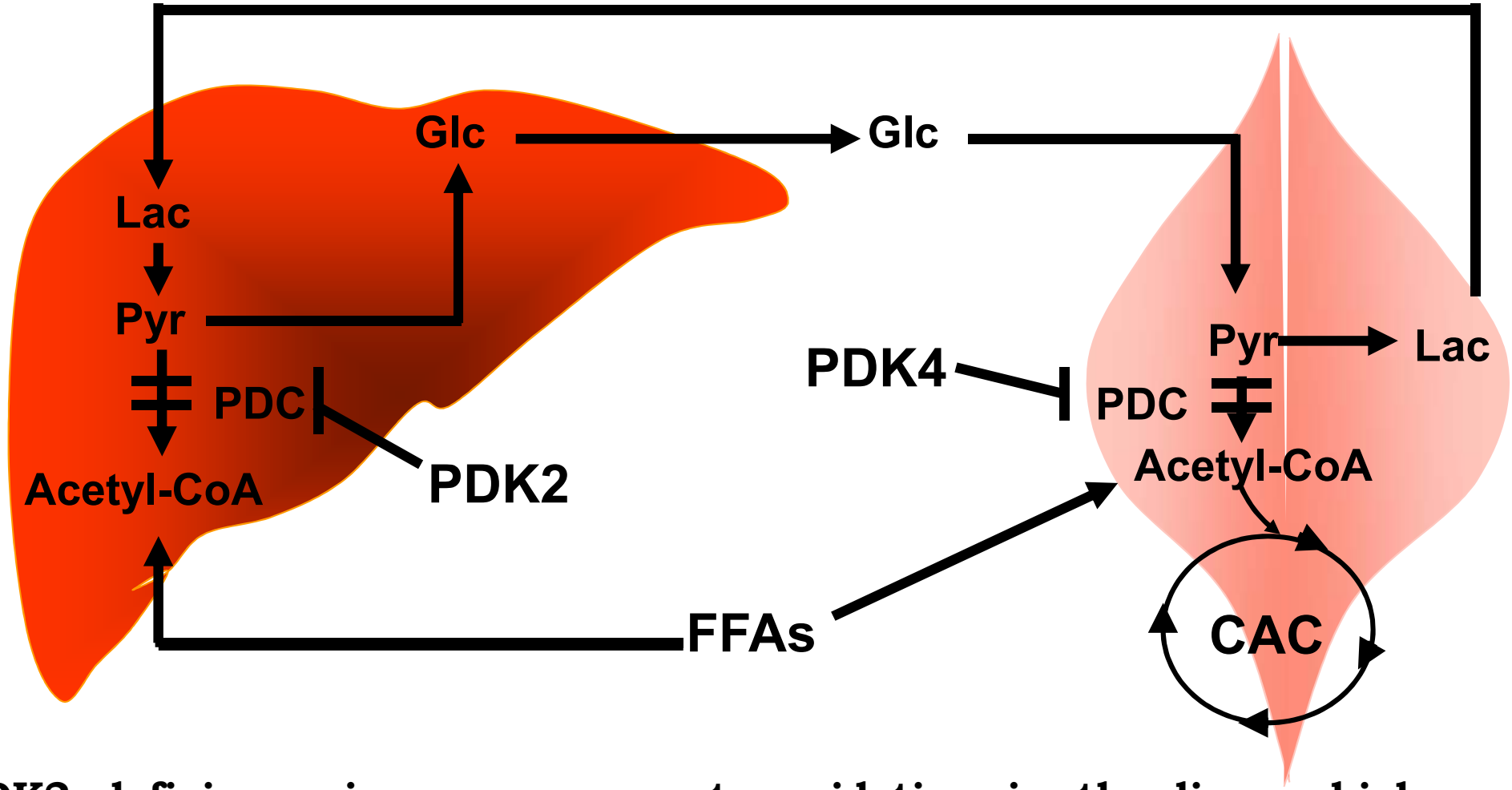
PDK2/PDK4 deficiency increases pyruvate oxidation and limits gluconeogenic substrates (pyruvate, lactate, alanine) which in turn limit the rate of glucose production resulting in low blood glucose levels.

Why are blood glucose levels reduced in PDK4 KO mice?



PDK4 deficiency increases pyruvate oxidation in peripheral tissues which reduces blood supply of gluconeogenic substrates (pyruvate, lactate, alanine) to the liver.

Why are blood glucose levels reduced in PDK2 KO mice?



PDK2 deficiency increases pyruvate oxidation in the liver which reduces the availability of pyruvate within the liver for glucose synthesis.

Summary

- **PDK deficiency lowers blood glucose by decreasing the availability of pyruvate for gluconeogenesis in the liver.**

- **In PDK4 KO mice, blood pyruvate and therefore liver pyruvate is reduced primarily by an increase in PDC activity in peripheral tissues.**

- **In PDK2 KO mice, liver pyruvate is reduced primarily by an increase in PDC activity in the liver.**

- **Regardless of this difference, PDK2 and PDK4 are potential therapeutic targets for diabetes.**