Regulation of Bioenergetics in PKD and CKD

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Aims

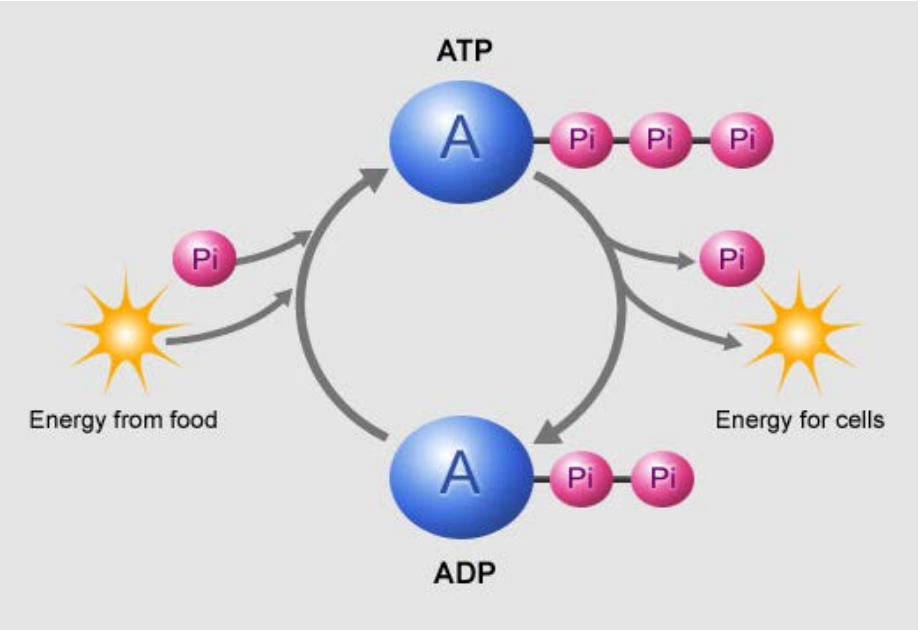
- To review what “cellular bioenergetics” means
- To discuss pathomechanisms which involve “metabolic switches”
- To highlight how to target metabolic pathways in kidney diseases using PKD as an example
- To discuss the role of reactive oxygen species and potential benefits of antioxidants

Energy

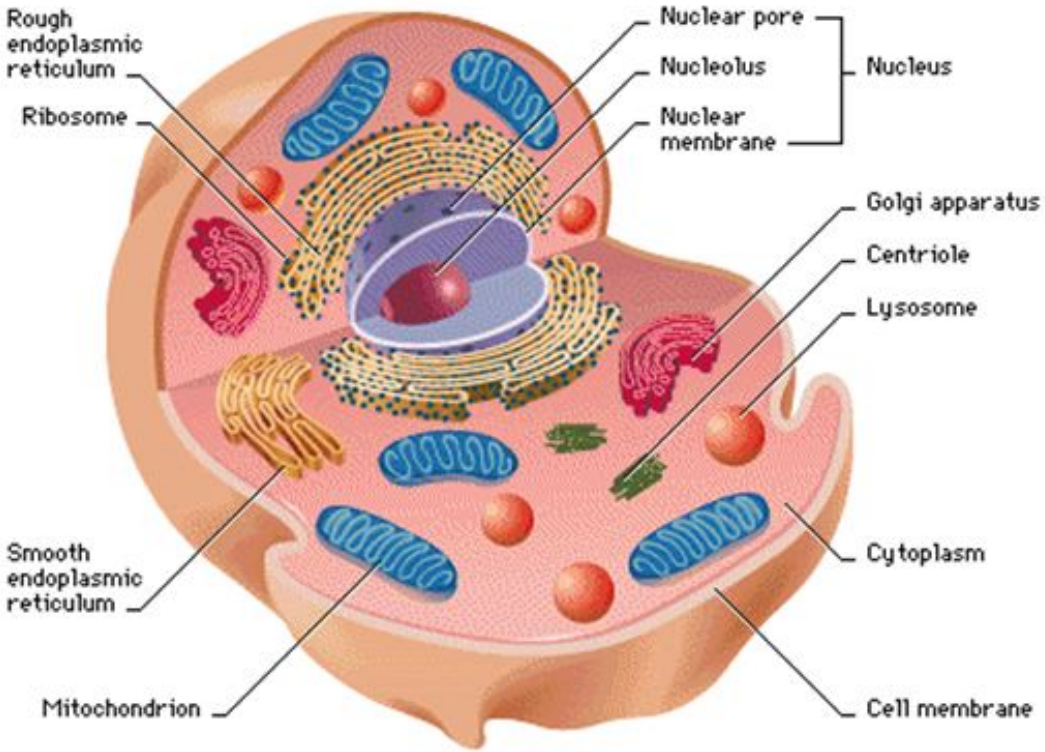
- **Everything we do**: having a thought or flexing a muscle, or digesting food **requires energy**.
- Our energy source is food but we need to convert it into a **renewable energy**, a molecule that can make the energy available for all of our cellular processes.

The energy currency of our cells is ATP

- A large part of the reason we **eat** and the reason why we **breath** is **to produce ATP** (adenosine triphosphate) in which high energy electrons store energy in chemical bounds.
- Each day our body “uses” up 60 kg of ATP in various cellular reactions, but at one time we have only 250 g.
- Food molecules are dismantled to provide the energy which is used to rebuild cellular ATP.

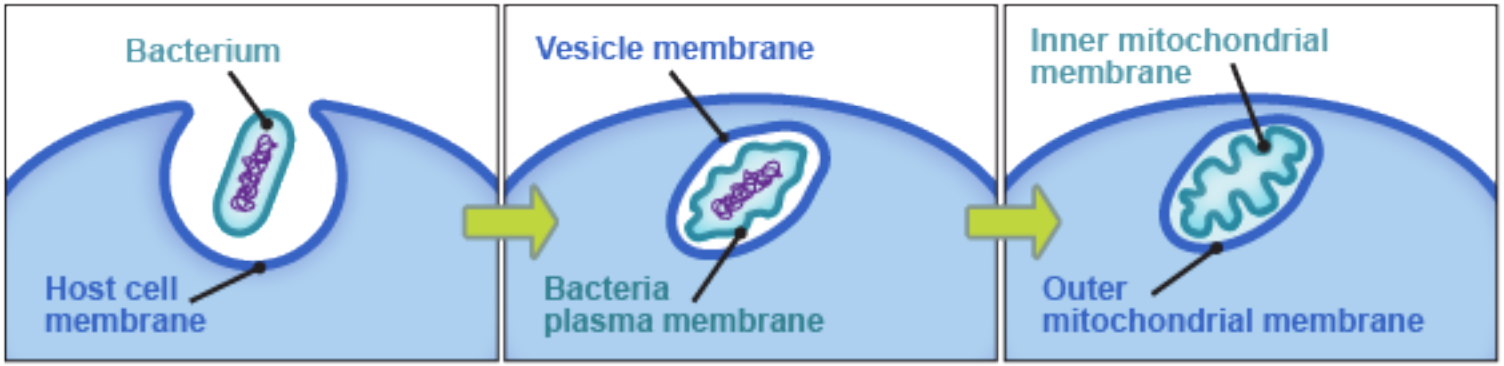


Mitochondria



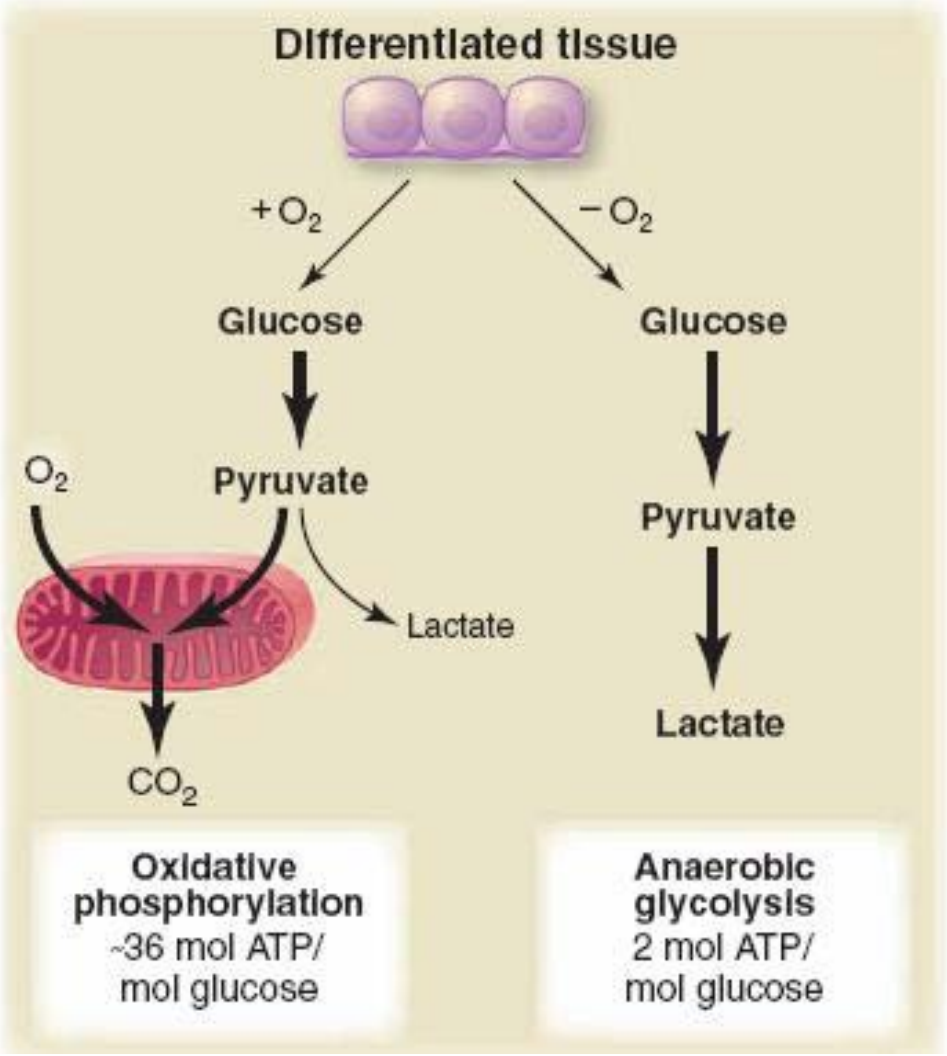
- **Mitochondria** convert the energy of food molecules into chemical energy of ATP. They are our “wind mills”.

Endosymbiosis theory



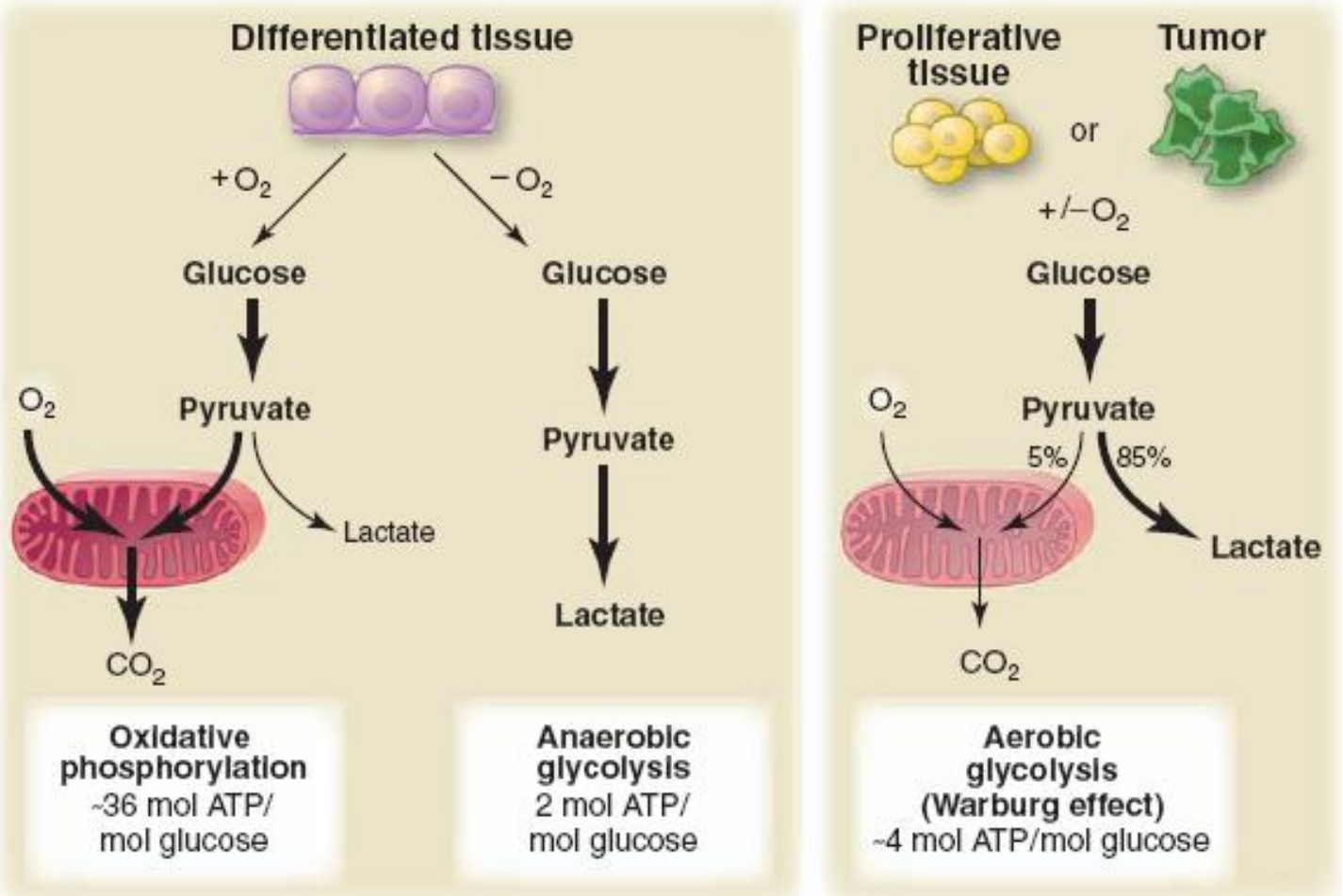
- Mitochondrion has the size of a bacterium
- Mitochondrial membrane: outer membrane is from host plasma membrane
- Mitochondrion has its own DNA, that is circular like bacterial DNA
- Mitochondria divide by fission

How can engulfing mitochondria benefit us? Aerobic *versus* anaerobic respiration



Vander Heiden *et al.* Science 2009

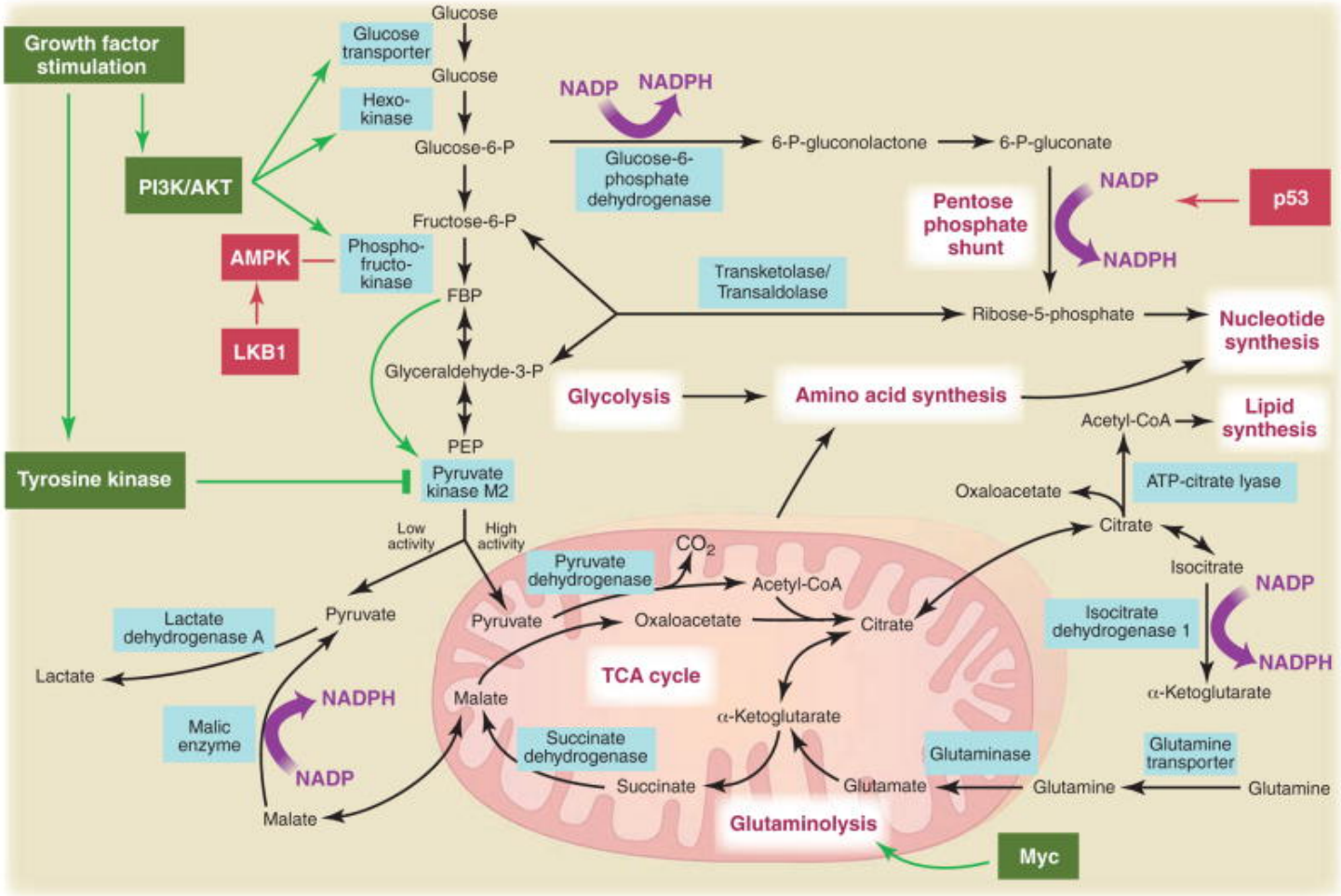
Metabolic differences between non-proliferating and proliferating cells: the Warburg effect



Vander Heiden *et al.* Science 2009

Otto Warburg Nobel Prize 1931

Effect of growth factor stimulation and tumor suppressors on cell proliferation



Vander Heiden *et al.* Science 2009

Clinical significance: Kidney diseases with proliferative component

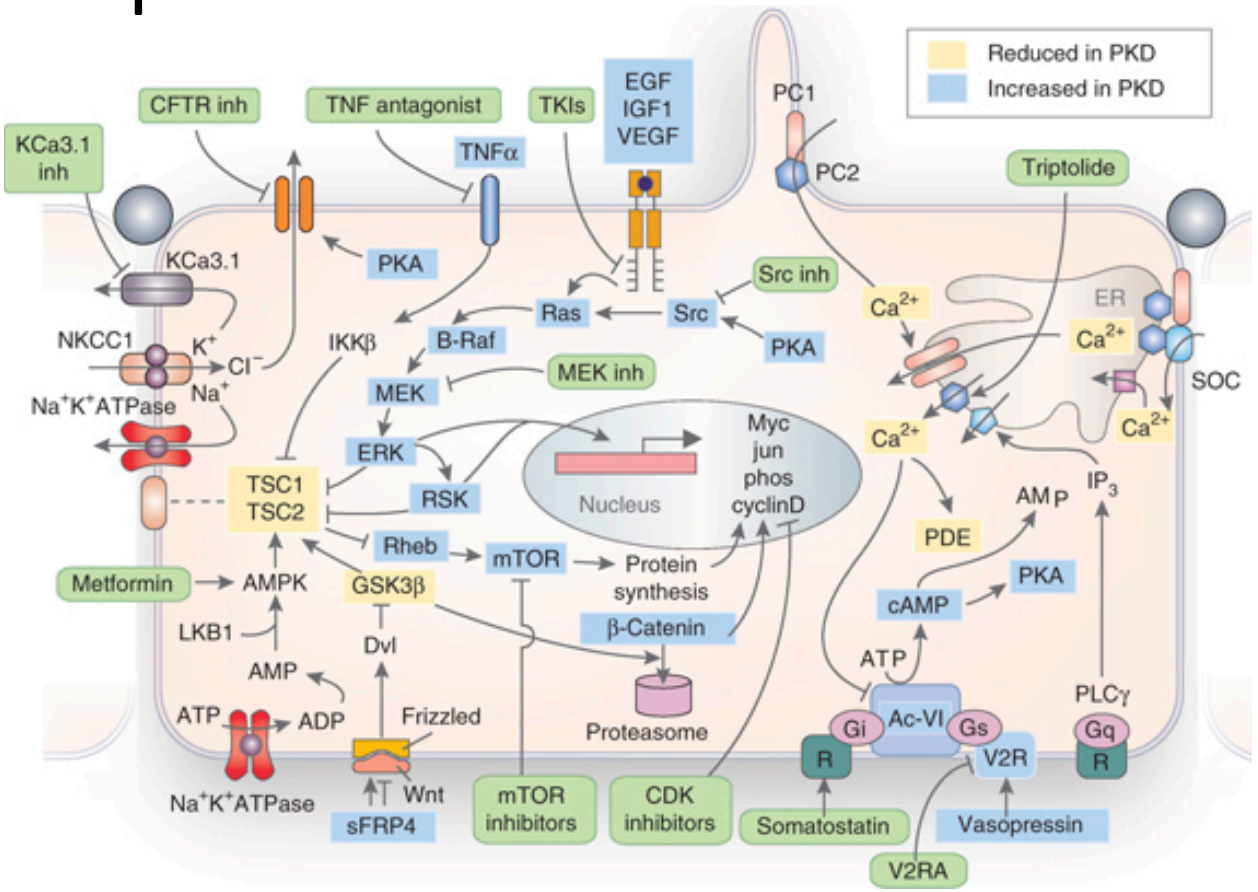
Understanding the mechanistic link between cellular metabolism and growth control can help us to better target proliferative diseases

- Kidney cancer (renal cell cancer: TCA cycle)
- Diabetic nephropathy (succinate)
- Glomerular proliferative diseases
- Cystic kidney diseases

Cystic kidney diseases

- One third of people older than 50 years develop renal cysts.
- Cysts develop from renal tubule segments. Cyst development is generally attributed to:
  - increased proliferation of tubular epithelium,
  - abnormalities in tubular cilia,
  - and excessive fluid secretion.
- Renal cystic disease has multiple etiologies.
- Most common: Autosomal dominant polycystic kidney disease (ADPKD) has an incidence of 1 per 400-1000 persons among whites and accounts for 8-10% of all cases of end-stage renal disease (ESRD).
- In acquired cystic renal disease, cysts are present in 8-13% of patients with chronic renal failure prior to dialysis. Following initiation of therapy, 10-20% of patients have acquired cystic renal disease after 3 years of dialysis, 40-60% after 5 years, and more than 90% after 10 years.

Signaling pathways involved in the pathomechanism of PKD

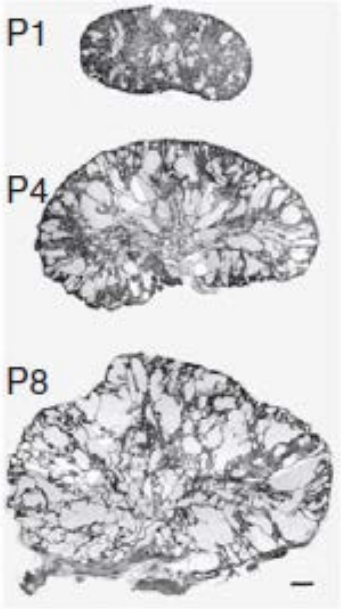


These signaling pathways are interconnected with metabolic pathways through receptor tyrosine kinases (EGFR), cell cycle proteins, sensors of cellular energy level (AMPK), oncogens (Src, Ras).

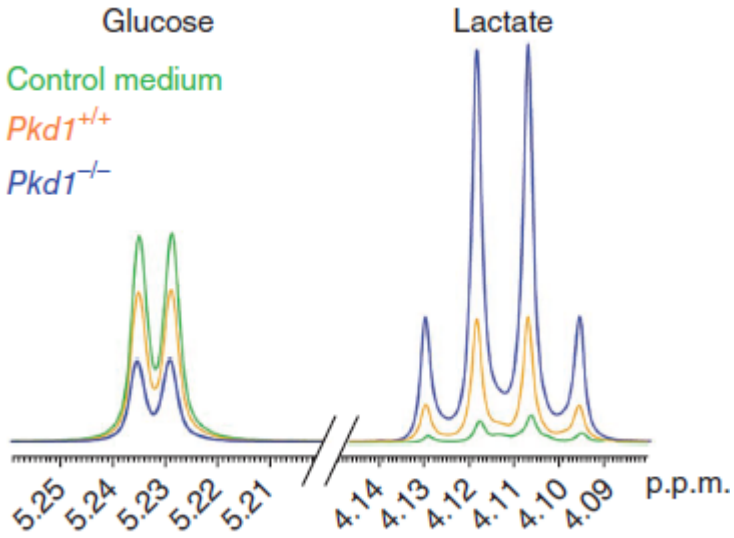
Torres V and Harris PC. *Kidney Int* 76: 149-68, 2009

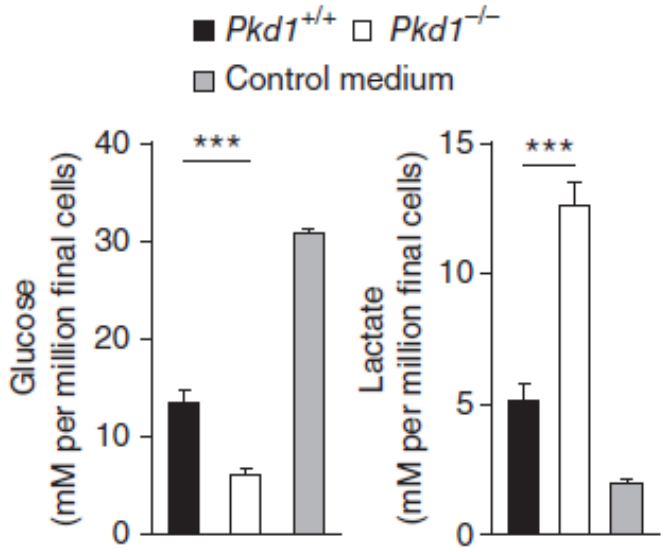
Increased cellular glycolysis drives increased cell proliferation in ADPKD (*Pkd1*$^{-/-}$)

Cyst growth



Glucose consumption and lactate production of cells



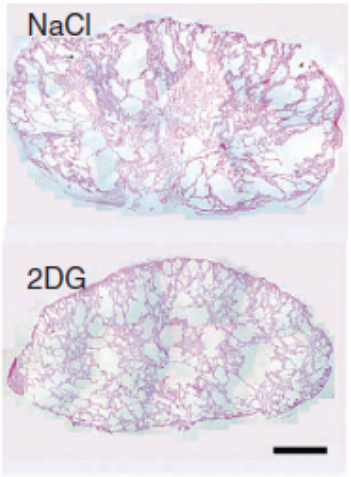


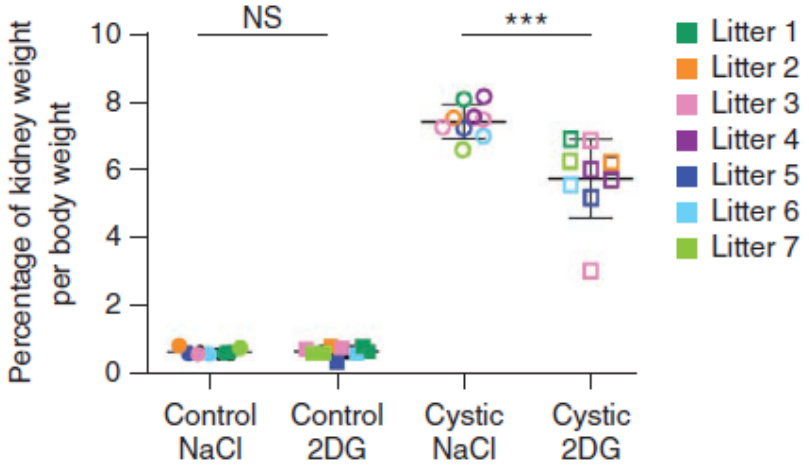
Rowe *et al.* Nature Medicine 2013

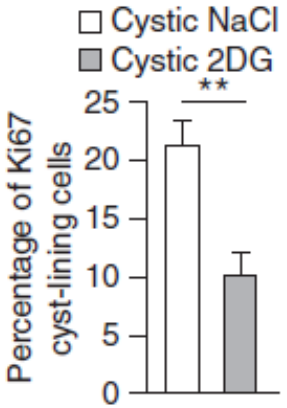
Nonmetabolizable analog of glucose (2-DG) inhibits cell proliferation and cyst growth

Cyst growth

Cell proliferation in cyst

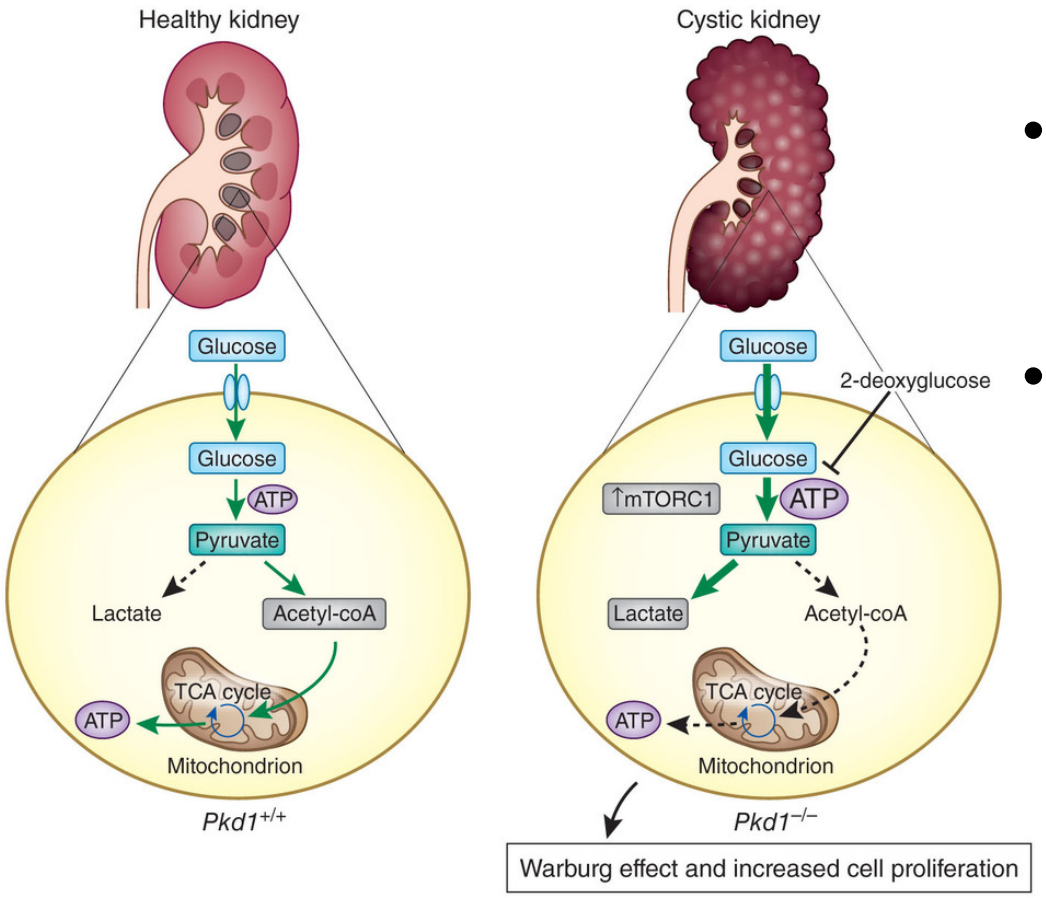






Rowe *et al.* Nature Medicine 2013

Metabolic switch drives cell proliferation in PKD



- 2DG can be a potential therapy in PKD by targeting glucose-addictive cells
- PKD as neoplastic disorder?

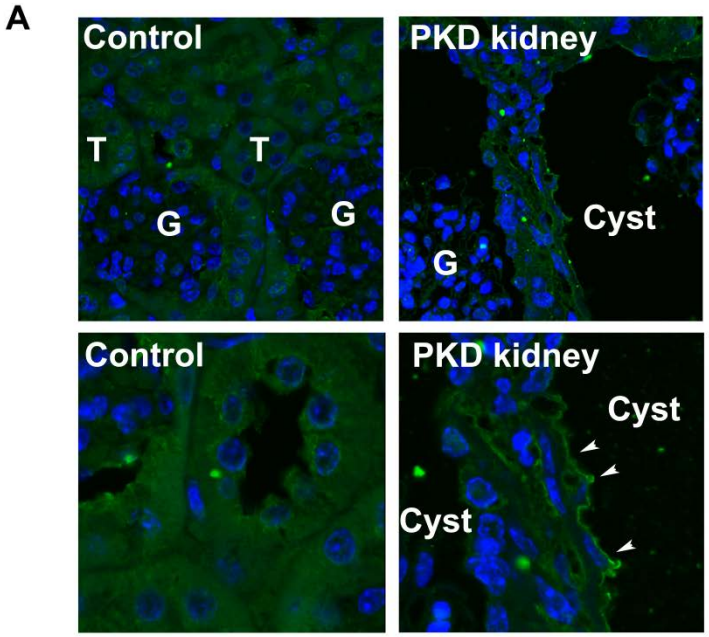
Priolo and Henske Nature Med 2013

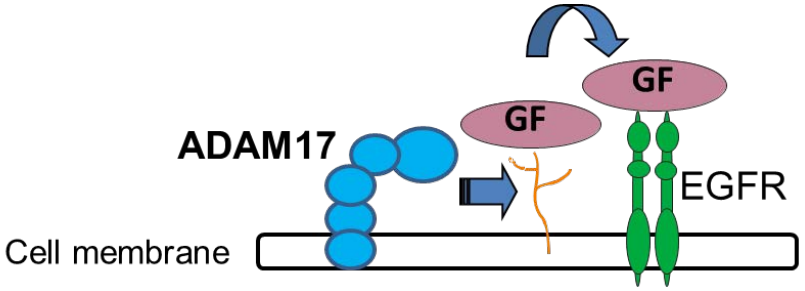
What causes the metabolic switch?

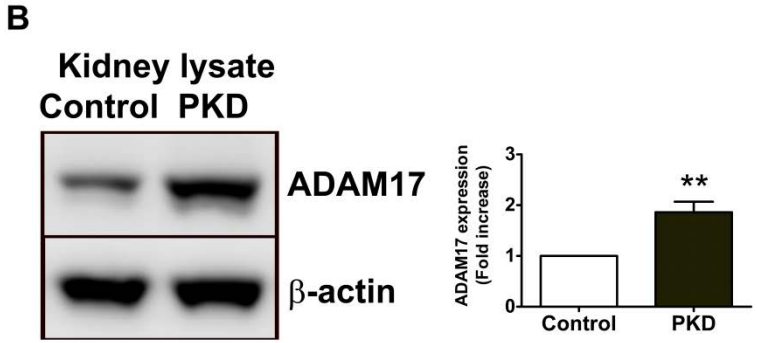
- Dysfunction of glycolytic enzyme (mutation, posttranslational modification?)
- Dysregulated cellular signaling mechanisms?

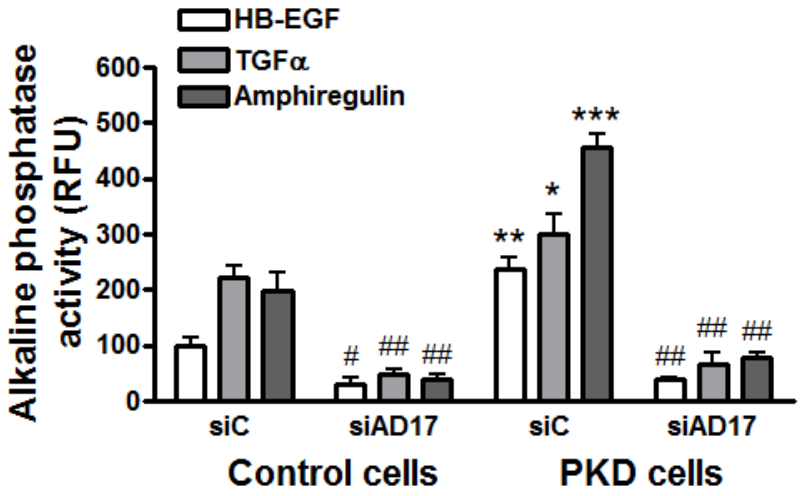
- Is similar metabolic switch present in other forms of PKD?
- And what happens to the mitochondria?

Increased ADAM17 expression and activity in ARPKD mice kidney



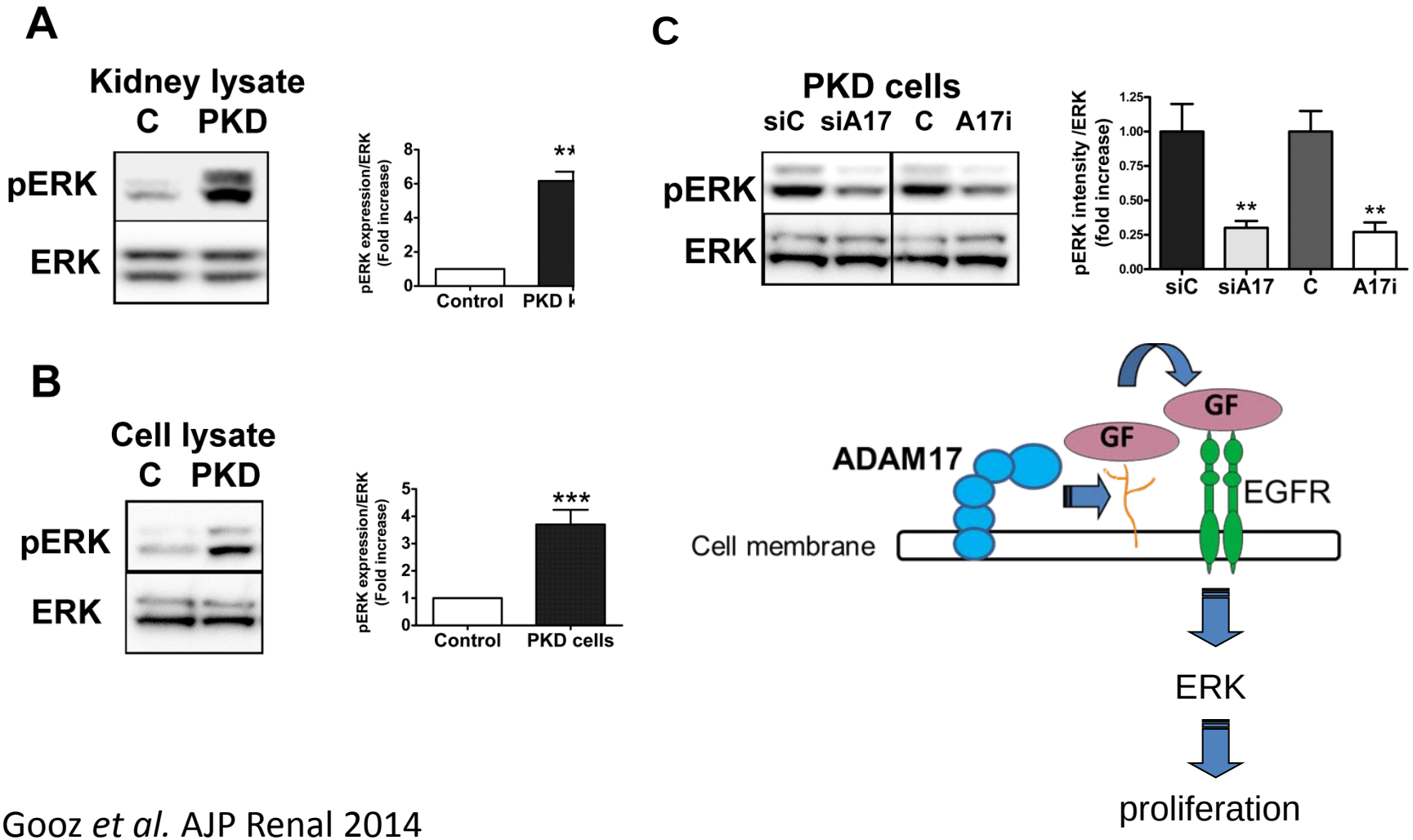






Gooz *et al.* AJP Renal 2014

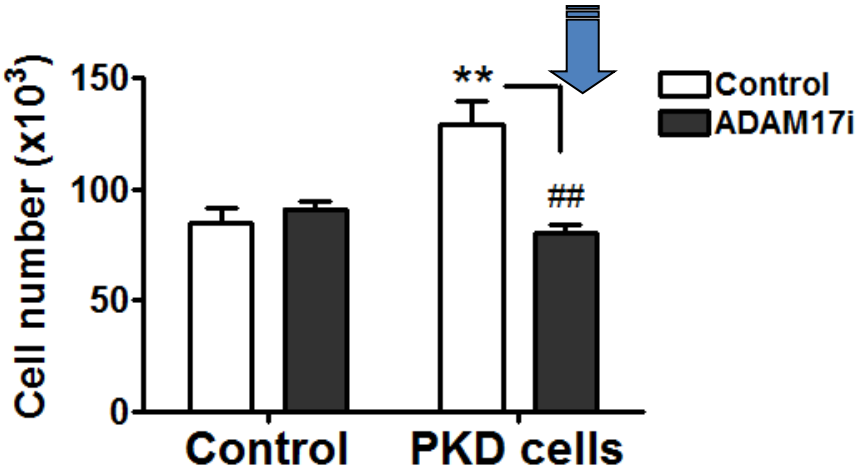
ADAM17 activity regulates cell proliferation through increased ERK activation in ARPKD



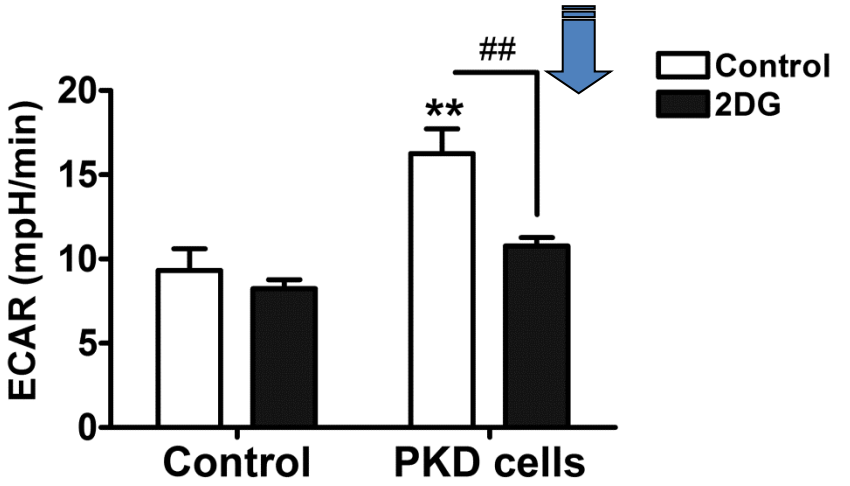
Gooz *et al.* AJP Renal 2014

ADAM17 activity regulates cell proliferation through increased glycolysis

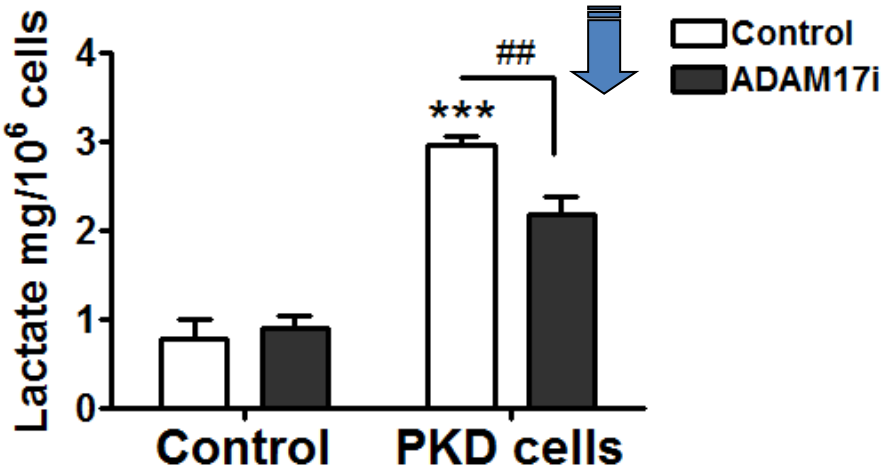
Proliferation assay

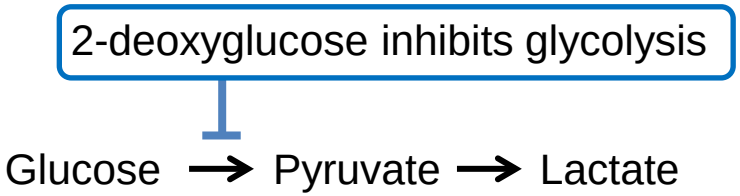


Seahorse: extracellular acidification rate



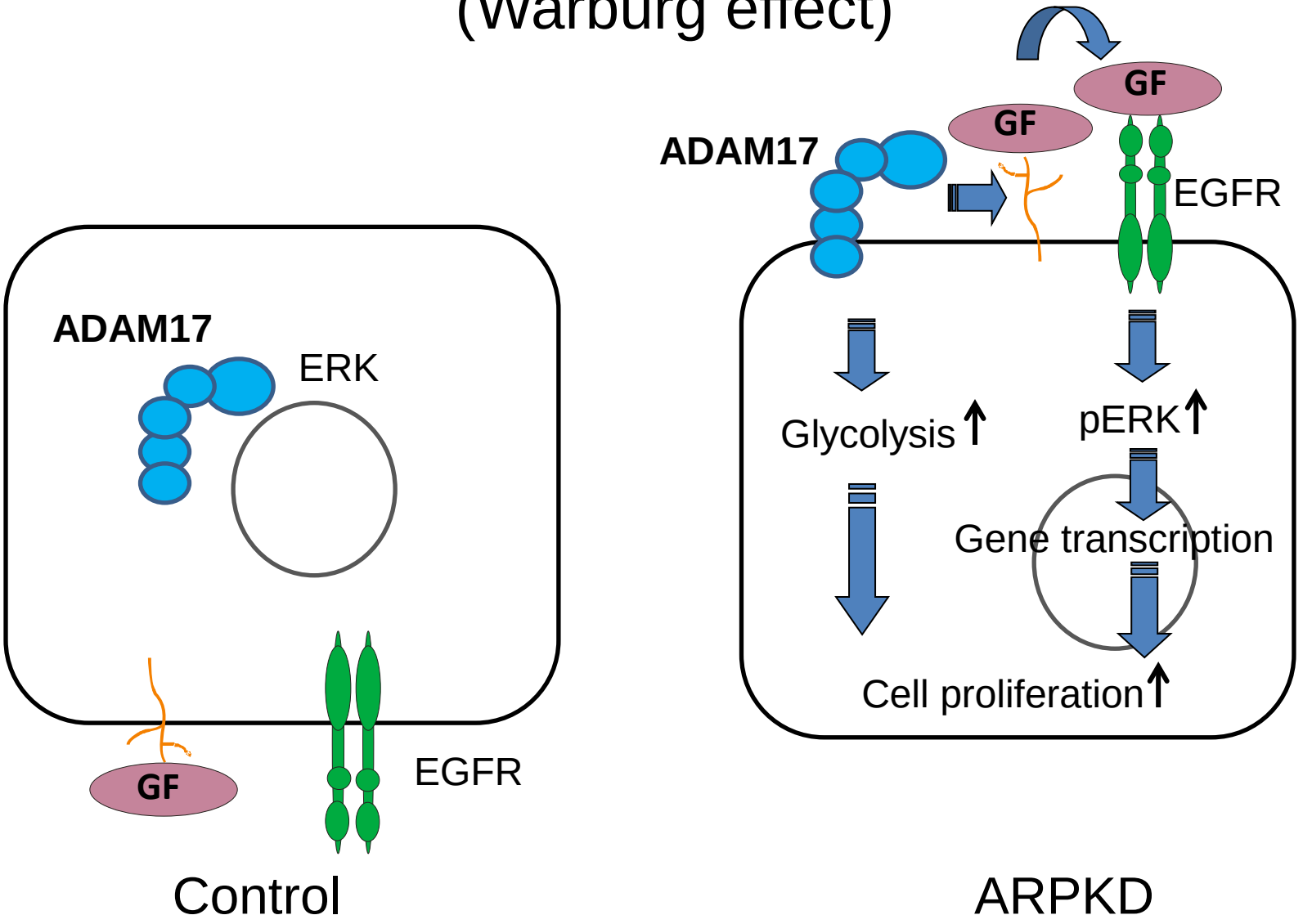
Lactate assay (colorimetric)





Gooz *et al.* AJP Renal 2014

ADAM17 promotes increased cell proliferation through EGFR/ERK activation and enhanced glycolysis (Warburg effect)



Gooz *et al.* AJP Renal 2014

Clinical significance of the data discussed

- Enhanced glycolysis is a feature of both ADPKD and ARPKD as well. This increases the potential **therapeutic value of 2DG**.
- 2DG is used for treatment of certain gliomas in as a combinational therapy. **No side effect of 2DG-treatment is reported** as of today.

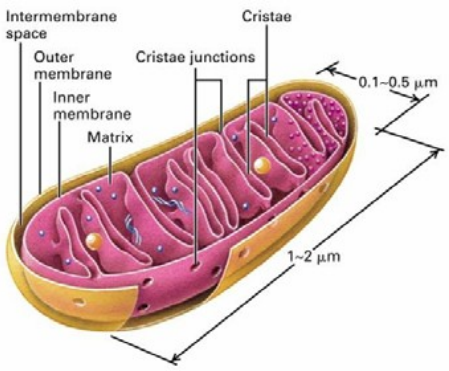
- **ADAM17 inhibitors** are in clinical trial for the treatment of cancer, rheumatoid arthritis and inflammatory bowel disease: one more reason to consider them for the treatment of PKD/ CKD.

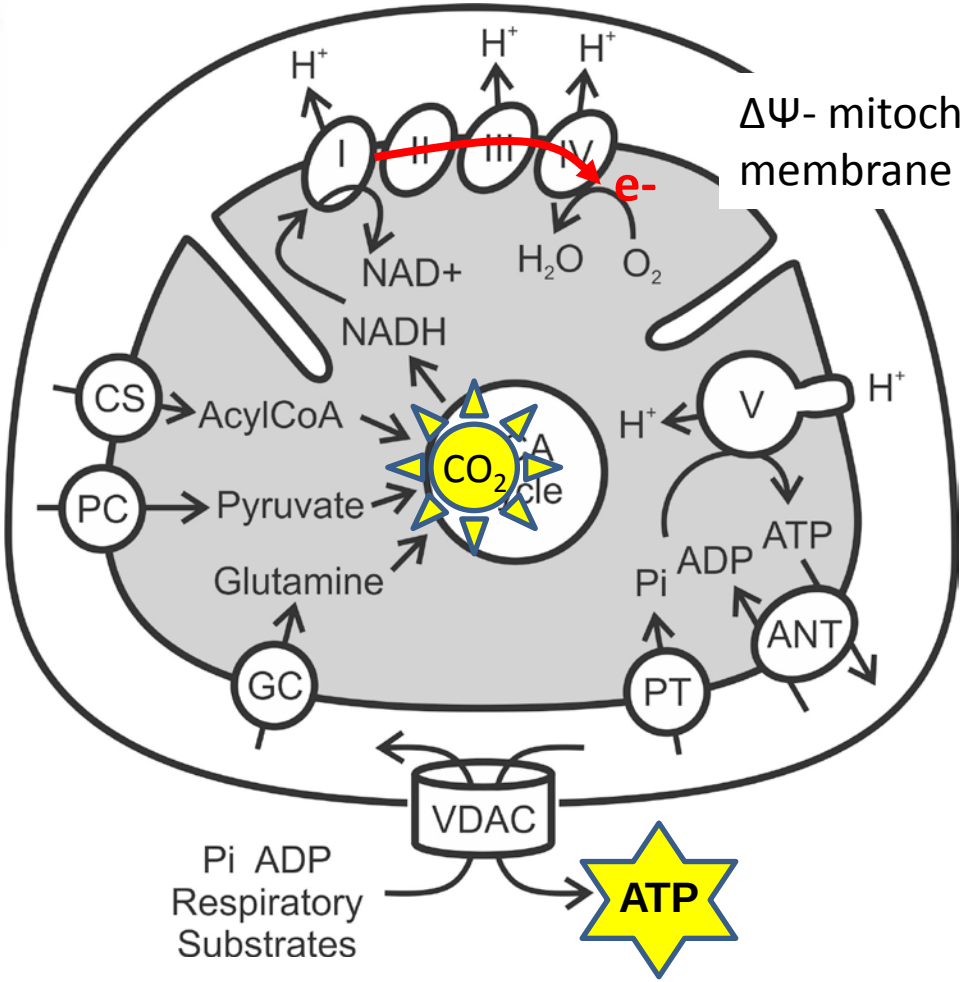
What happens in the mitochondria during metabolic switch?

Mitochondria Dysfunction and CKD

- Renal cell carcinoma (Junker *et al.* PLOS One 2011)
- Angiotensin-induced oxidative stress (Sun *et al.* AJP Renal 2010)
- Diabetes (Sengupta *et al.* PLOS One 2009,)
- Alterations in respiration gene expression in the white blood cells of CKD patients (Granata *et al.* BMC Genomics, 2009)

Electron transport chain and oxidative phosphorylation





ΔΨ- mitochondrial membrane potential

Maldonado *et al.* JPET 2012 *modified*

Reactive oxygen species (ROS)

- ROS are the strongest stimulator of UCP2 protein production
- ROS are chemically reactive molecules which contain oxygen (O_2^-, H_2O_2)
- Mostly produced in mitochondria
- Required for normal cellular signaling
- In excess:
  - Damage cellular DNA and lead to mutation
  - Increase proliferative pathways (cancer)
  - Cause cellular damage, aging, apoptosis, death

Antioxidant therapy

- Antioxidants: Cellular enzymes (SOD, catalase, glutathione peroxidase), small molecules (Vitamin A, E, glutathione), uric acid, polyphenol.
- Antioxidants did not work in clinical trial for the treatment of PKD
- Results with antioxidant therapy are inconclusive and contradictory
- Reason:
  - Difficult interactions between antioxidant enzymes
  - Antioxidants can have pro-oxidant features
- ROS-elevating and/or ROS-eliminating strategies can both work for cancer treatment, similar is expected in CKD/PKD.

Summary

- Metabolic switch is the feature of highly proliferative cell types
- Advantages of metabolic switch (Warburg effect):
  - To escape oxidative damage
  - To provide enough ATP
  - To produce “biomass” for new cell production
- To effectively target CKD/PKD the antioxidant/pro-oxidant strategy needs to be combined with targeting the metabolic pathways that support proliferation in the disease.

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