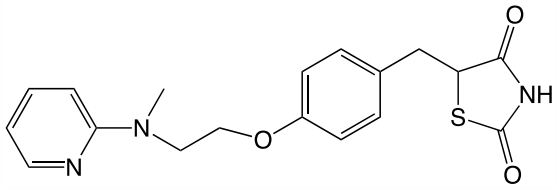
Combining Seahorse XF analysis with stable isotope tracing to reveal novel drug targets for metabolic and neurodegenerative disease

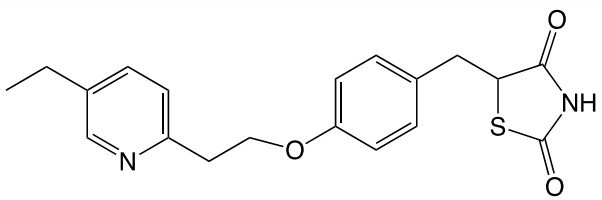
Ajit Divakaruni, Ph.D.
July 20, 2017
Asst. Professor of Molecular & Medical Pharmacology, UCLA

Thiazolidinediones (TZDs)

Rosiglitazone (Avandia®)



Pioglitazone (Actos®)

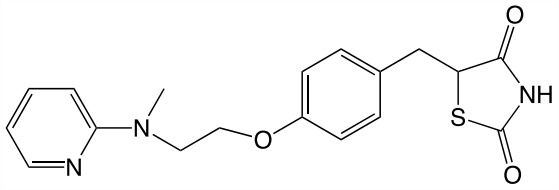


Effective insulin sensitizers with prohibitive side effects

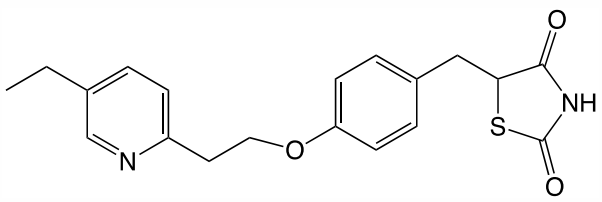
Is PPAR*γ* agonism the only mechanism of action?

Thiazolidinediones (TZDs)

Rosiglitazone (Avandia®)



Pioglitazone (Actos®)



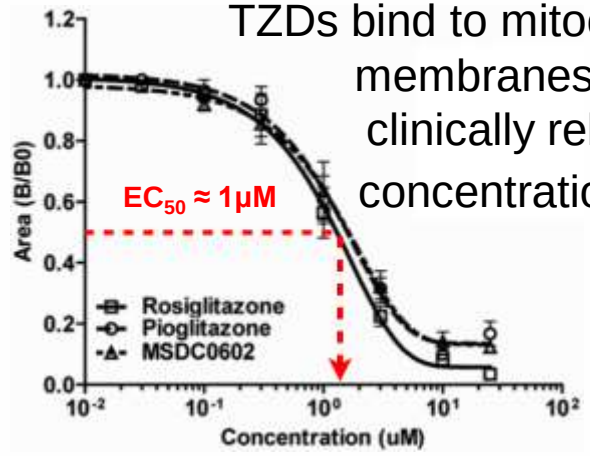
Effective insulin sensitizers with prohibitive side effects

Is PPARγ agonism the only mechanism of action?

Rapid *in vitro* and *in vivo* effects

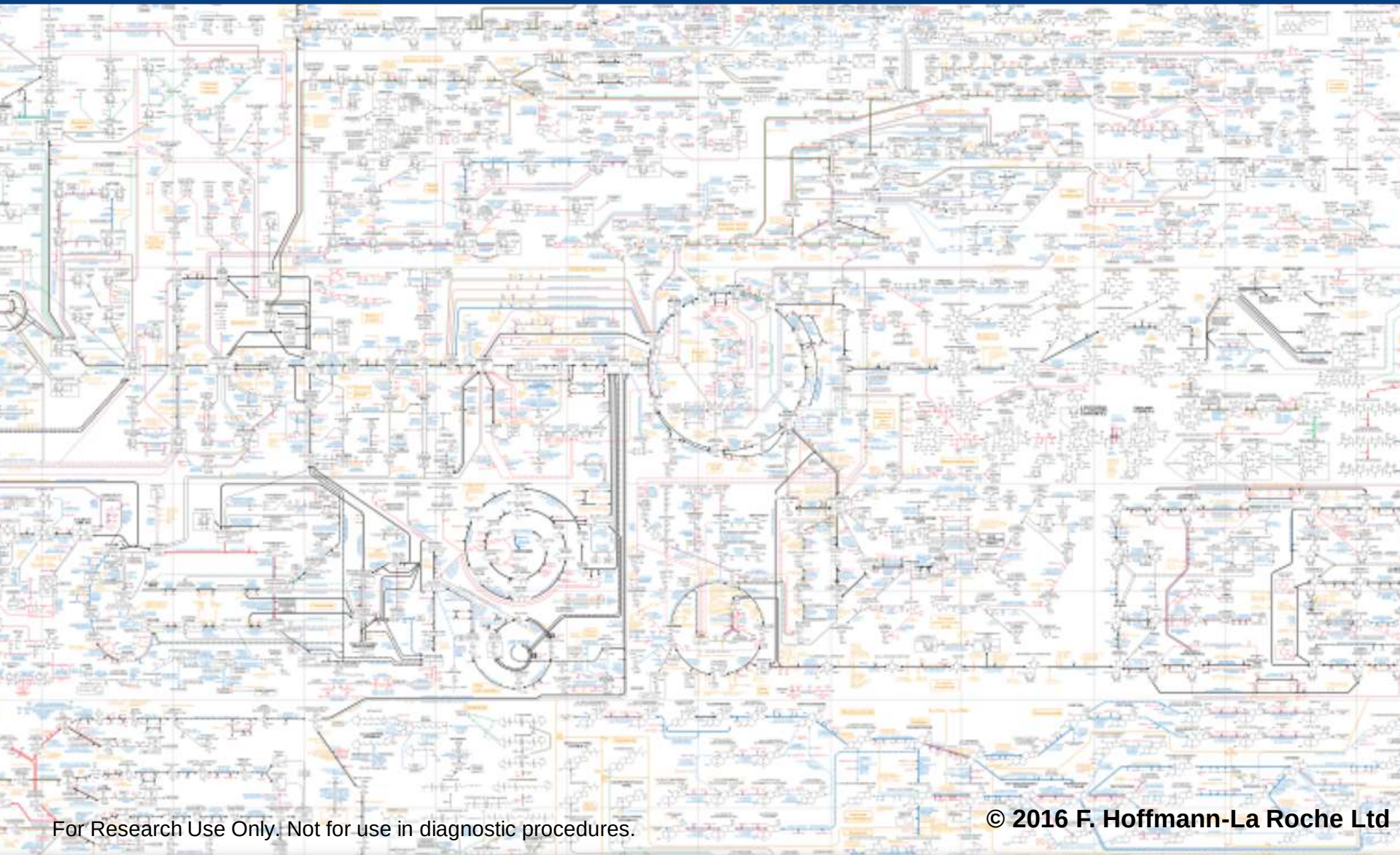
An insulin-sensitizing effect persists in some tissue-specific KO models

TZDs bind to mitochondrial membranes at clinically relevant concentrations



Chen…and Finck (2012) J. Biol. Chem.

Metabolism



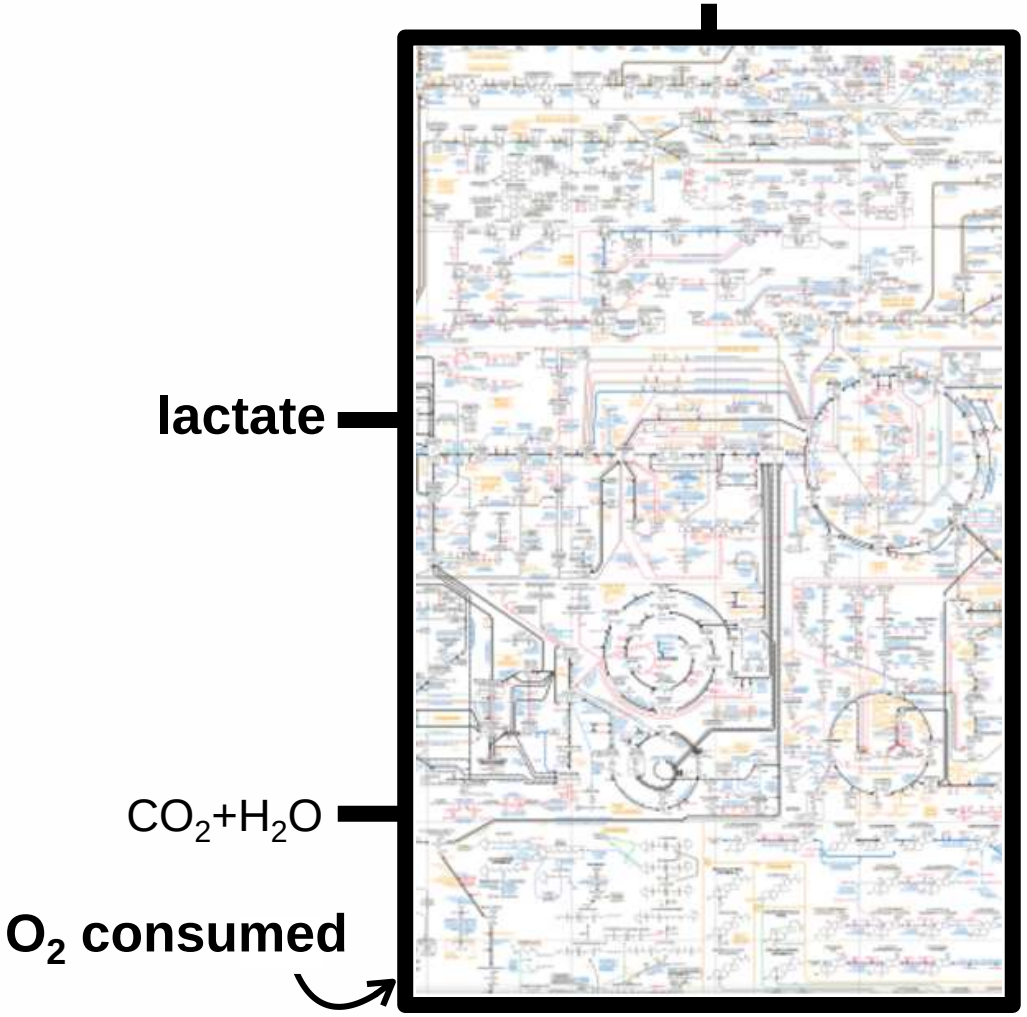
A “black box” approach

(1) MEASURE RATES OF INPUTS AND OUTPUTS

(Respiration, lactate efflux)

Quantitative flux through an intact network

Real-time rates upon acute perturbations



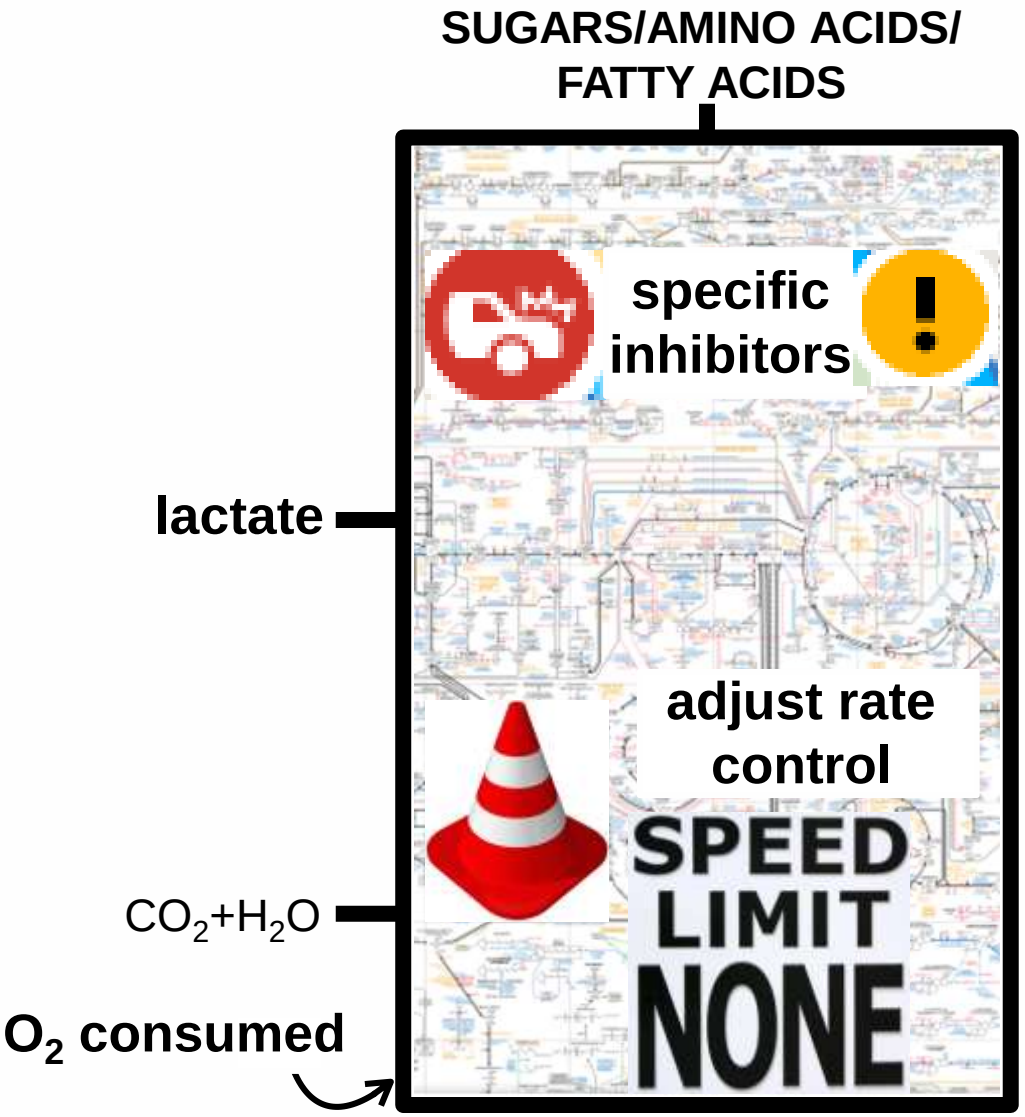
A “black box” approach

(1) MEASURE RATES OF INPUTS AND OUTPUTS

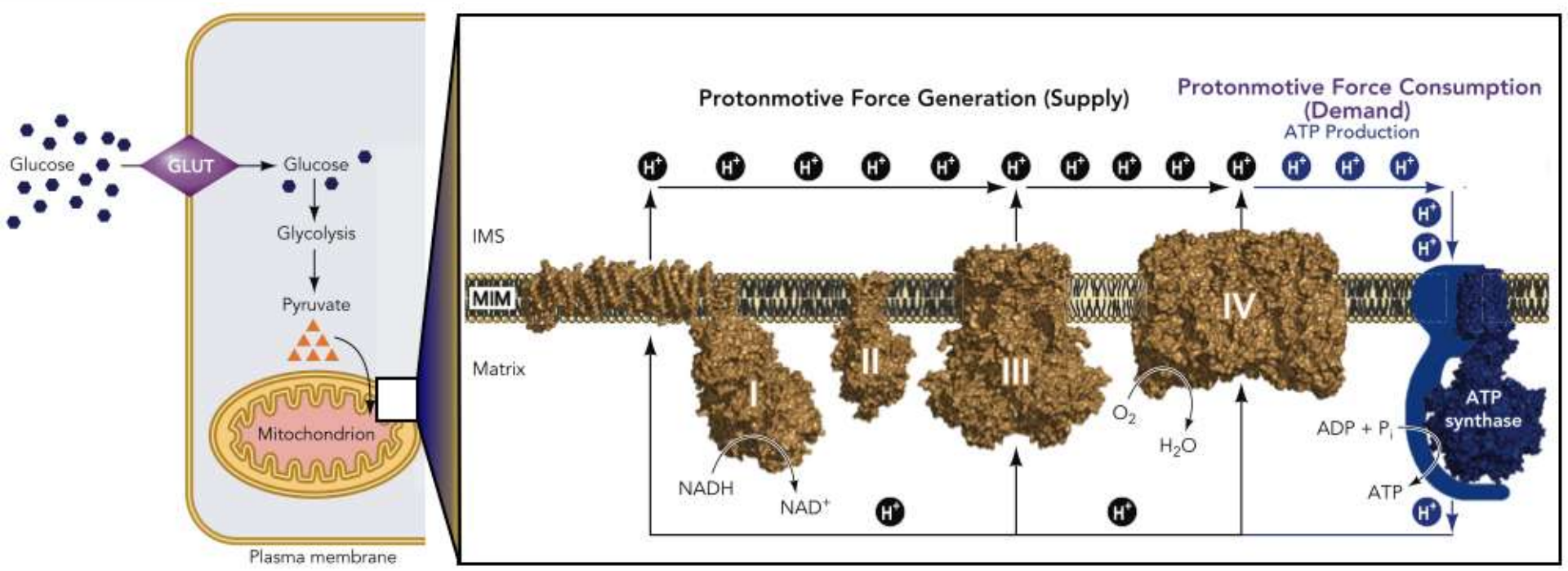
(Respiration, lactate efflux)

Quantitative flux through an intact network

Real-time rates upon acute perturbations

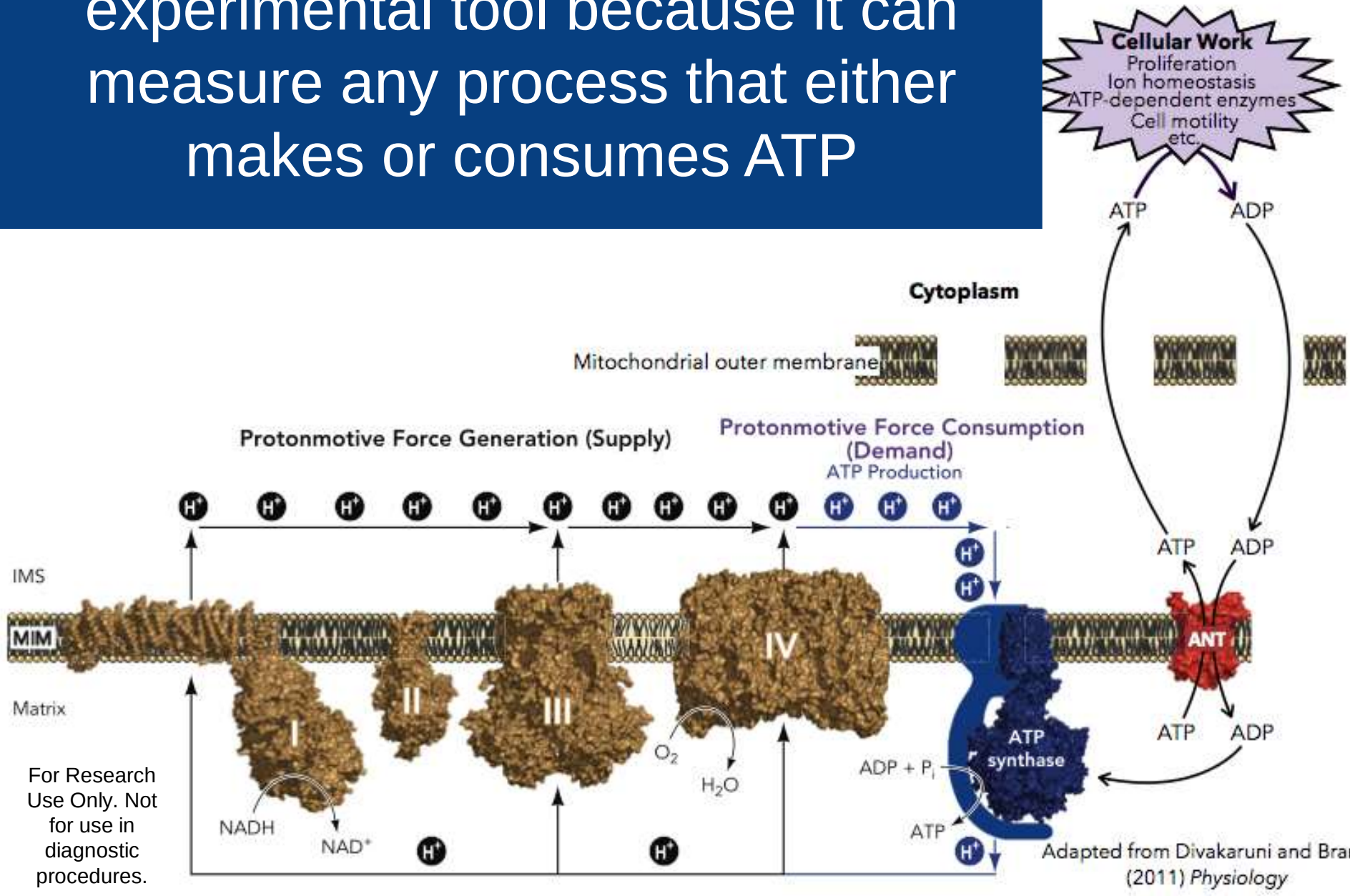


Respiration is a powerful experimental tool to study mitochondrial metabolism



Adapted from Divakaruni and Brand (2011) *Physiology*

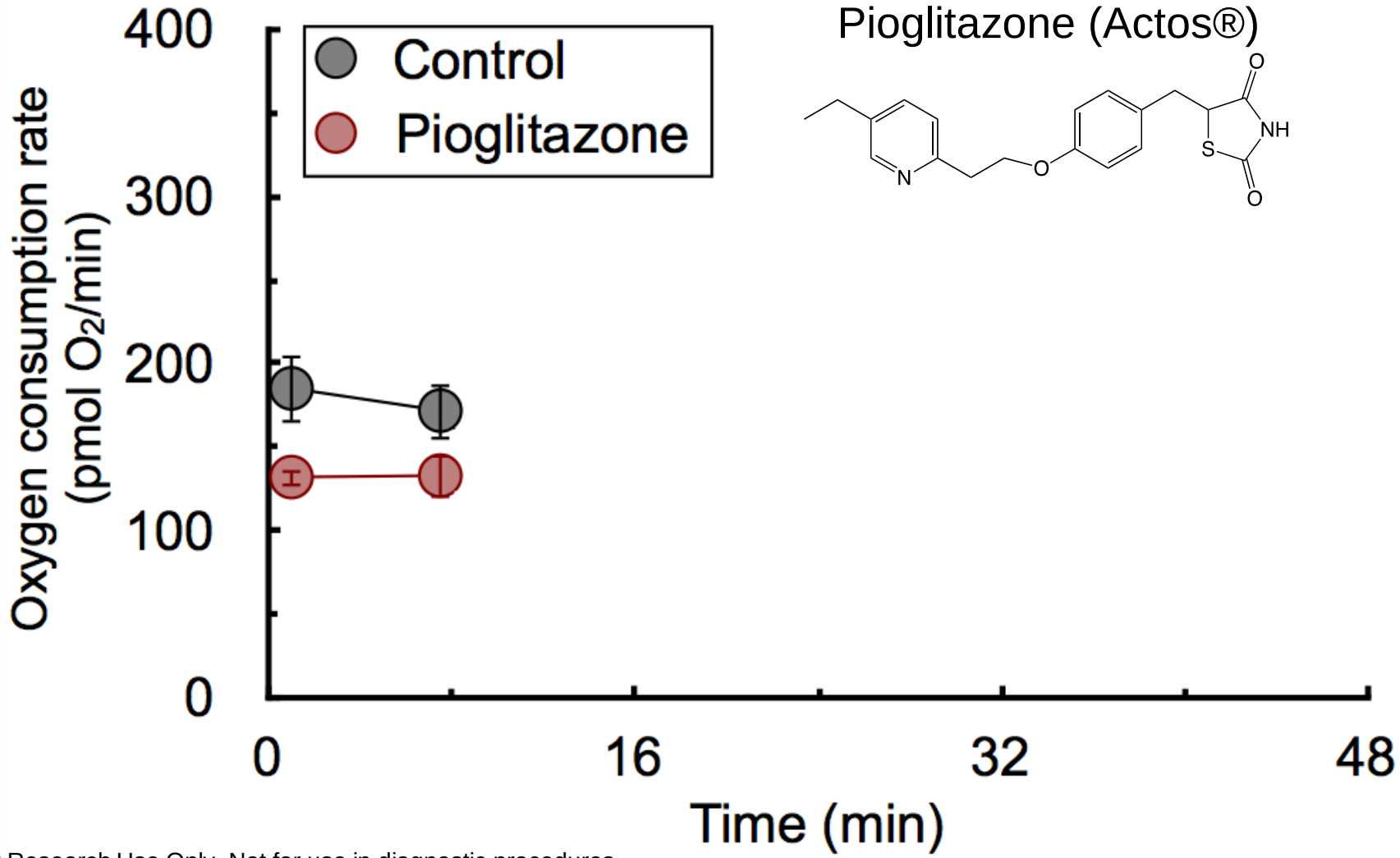
Respiration is a powerful experimental tool because it can measure any process that either makes or consumes ATP



Adapted from Divakaruni and Brand (2011) *Physiology*

Is there a direct effect of TZDs on mitochondrial function?

RESPIRATION IN CULTURED MYOCYTES

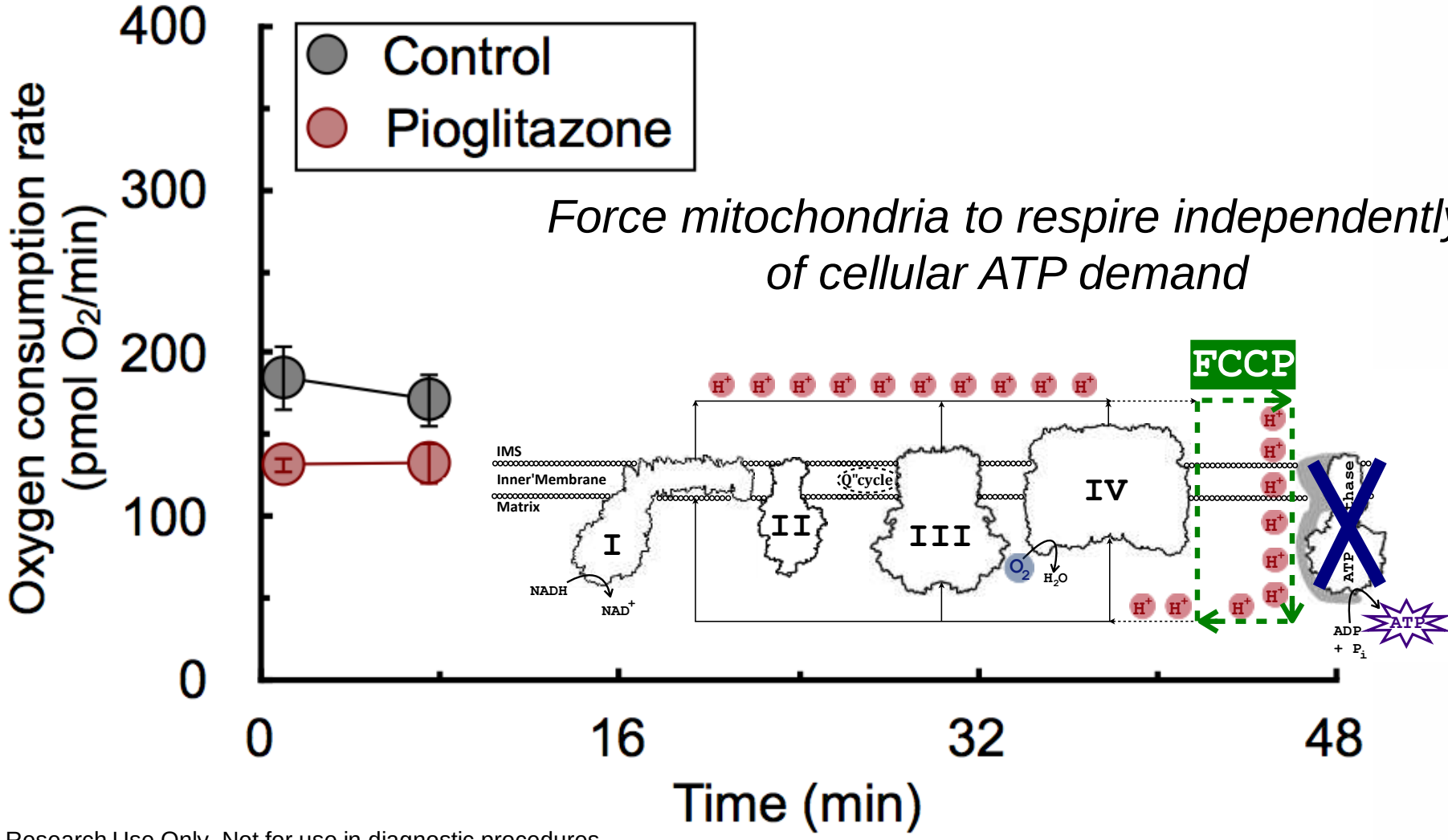


Pioglitazone (Actos®)

see Divakaruni…and Jastroch (2014) Meth. Enzym.

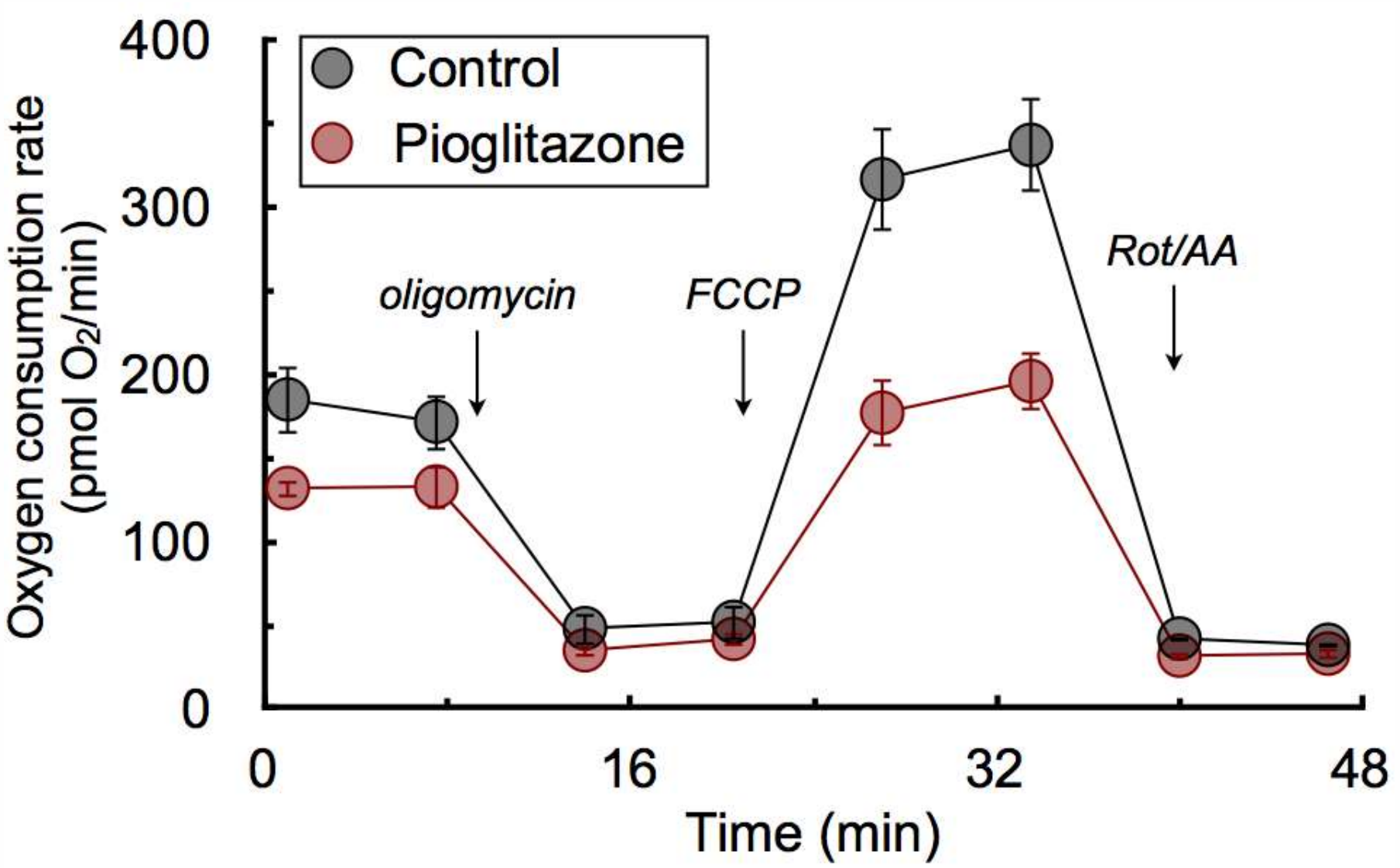
Is there a direct effect of TZDs on mitochondrial function?

RESPIRATION IN CULTURED MYOCYTES



see Divakaruni…and Jastroch (2014) Meth. Enzym.

Pioglitazone has a direct, acute effect on mitochondrial function



see Divakaruni…and Jastroch (2014) Meth. Enzym.

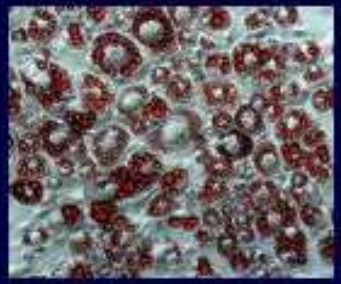
Decreased respiratory capacity could be due to dozens of possibilities

•SUBSTRATE/ION TRANSPORTERS

•GLUT •ARALAR •MCU •MCT •Plasma mem./mito. amino acid transport •CACT
•Mito. pyruvate carrier •Mito. dicarboxylate carrier •NCX •Mal./asp. shuttle •CD36

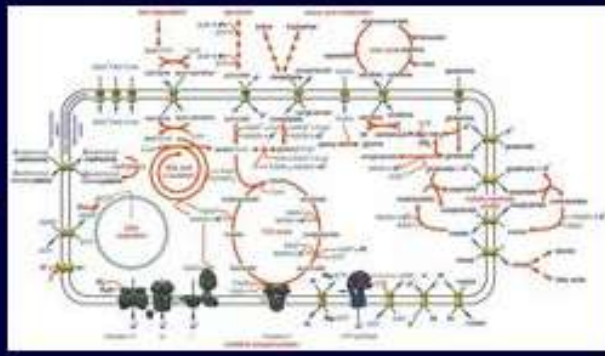
•MOBILIZATION OF INTERNAL ENERGY STORES

•Glycogenolysis •Endogenous lipolysis •Oxidation of endogenous amino acids



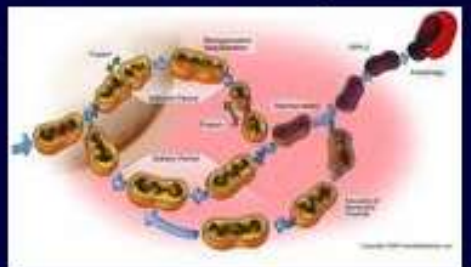
•ACTIVITY OF RATE-CONTROLLING ENZYME

•Respiratory complex I •PDH •CPT-1 •Hexokinase •αKGDH •β-oxidation enzymes
•ETFDH •Respiratory complex III •BCKDH •SDH •Aconitase •MDH •FH •CPT-2
•Ketone body oxidation enzymes •GAPDH •Citrate synthase •Cytochrome oxidase
•IDH •Glutaminase •GDH •Cytochrome c •AsAT •G3PDH •AlAT •BCAT



•MITOCHONDRIAL ULTRASTRUCTURE

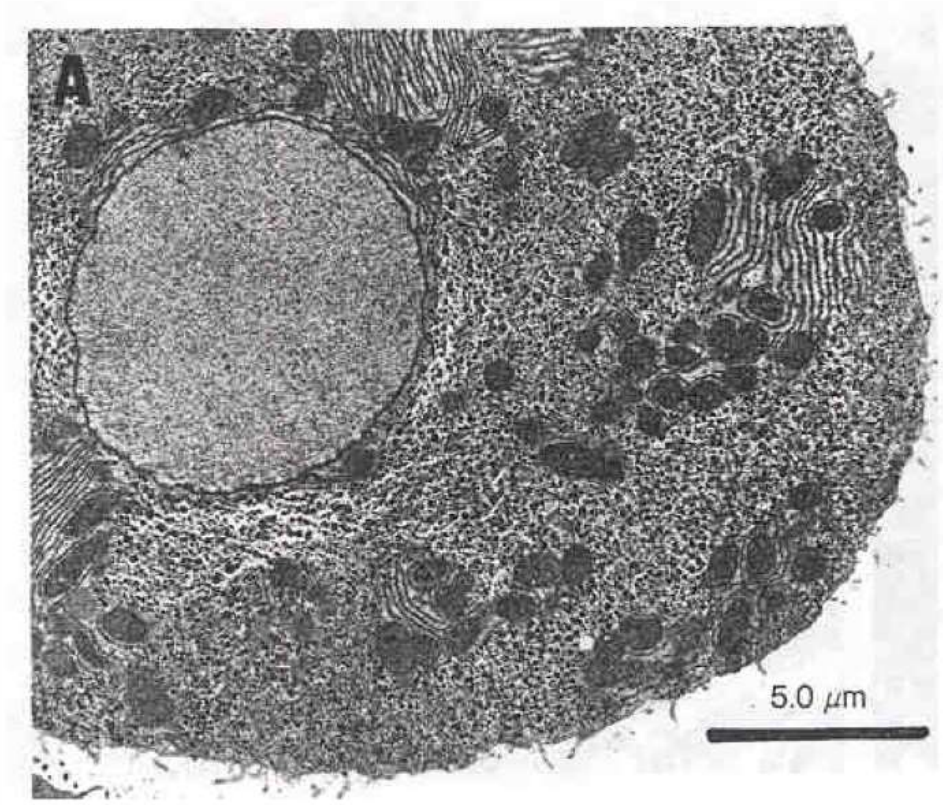
•Content •Volume •Cristae density •Turnover •Fusion/Fission

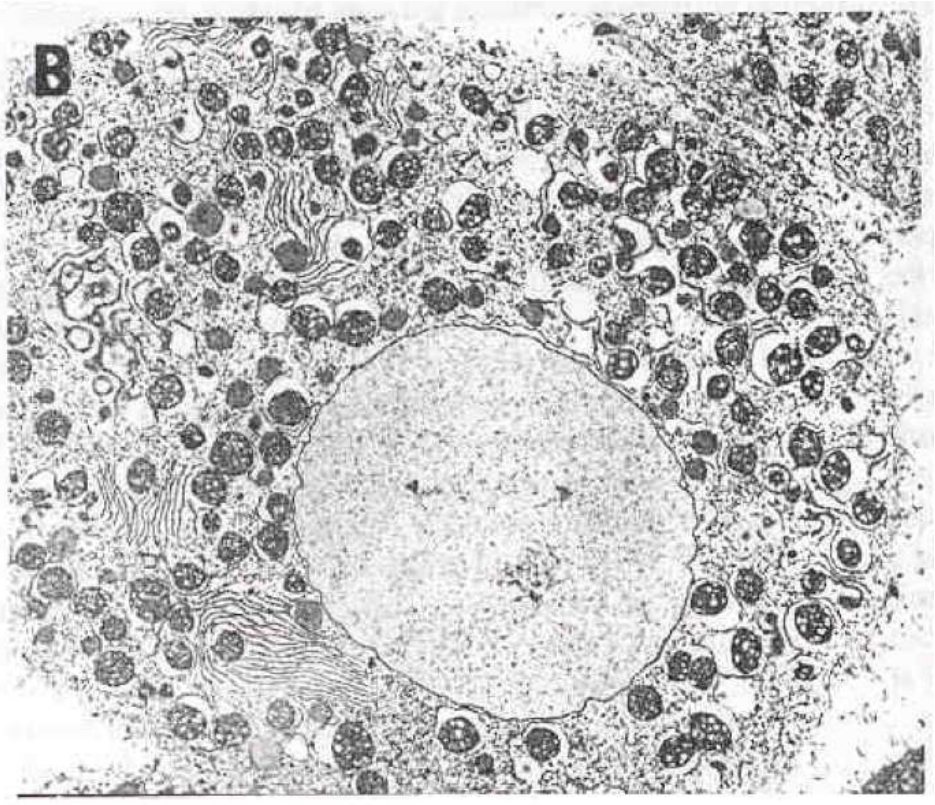


Permeabilized cells provide control over mitochondrial substrate provision

Intact cell

Permeabilized cell

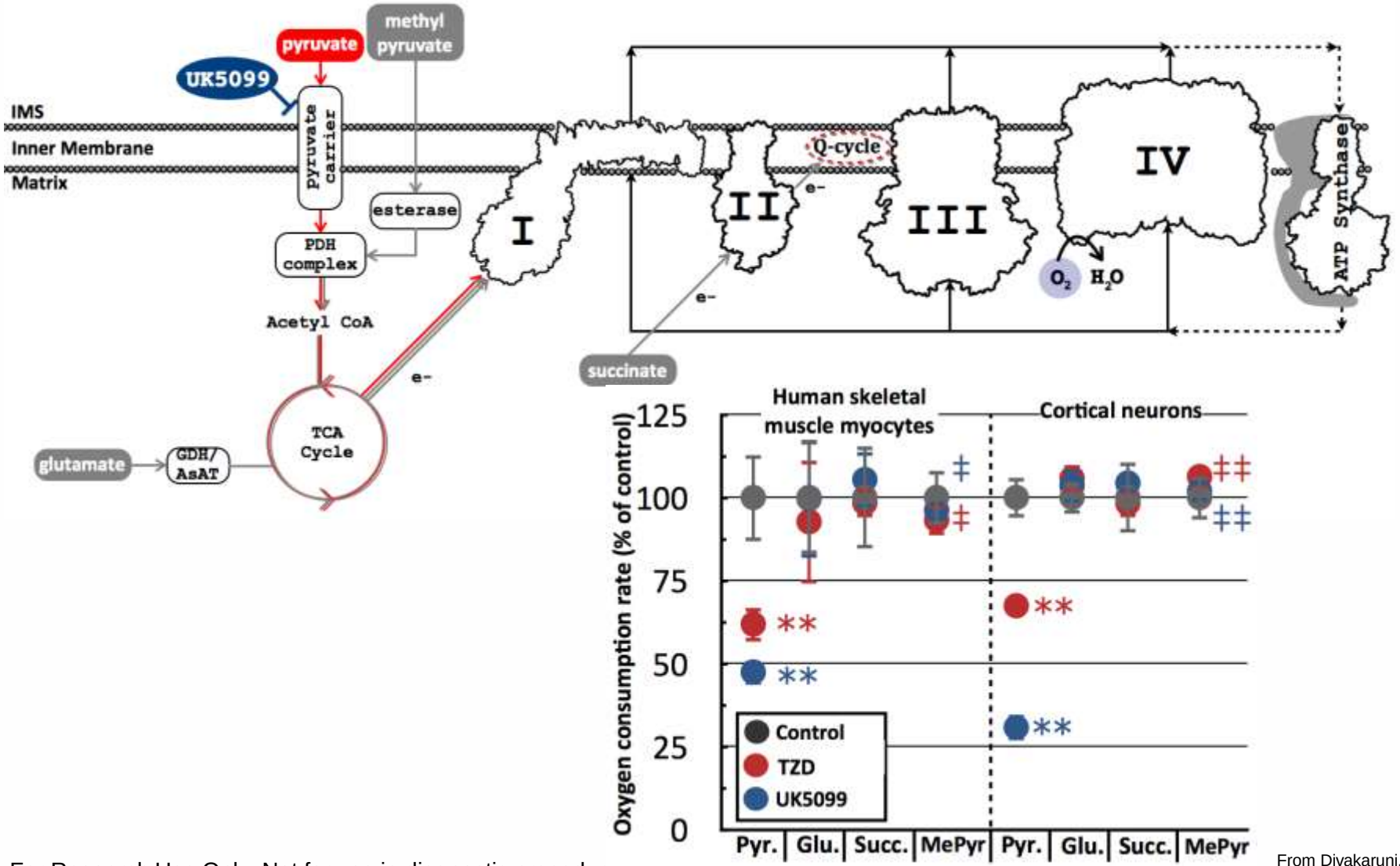




Validation in Divakaruni...and Murphy (2013) *PNAS;*
Protocols in Divakaruni…and Murphy (2014) *Curr. Prot. Tox.*

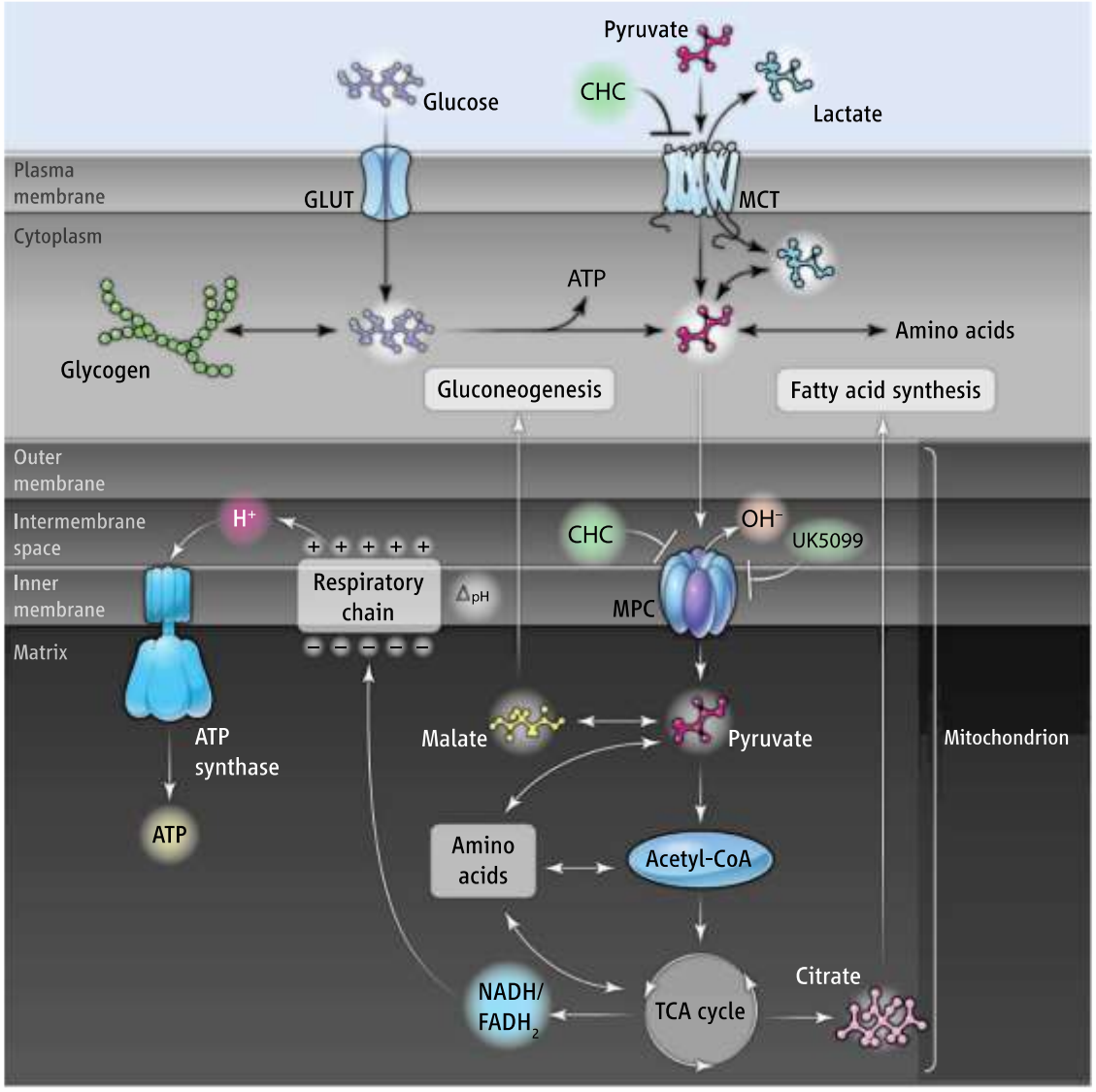
Image from Fiskum…and Lehninger (1980) *PNAS*

TZDs acutely inhibit mitochondrial pyruvate uptake



From Divakaruni...and Murphy (2013) *PNAS*

The mitochondrial pyruvate carrier (MPC)



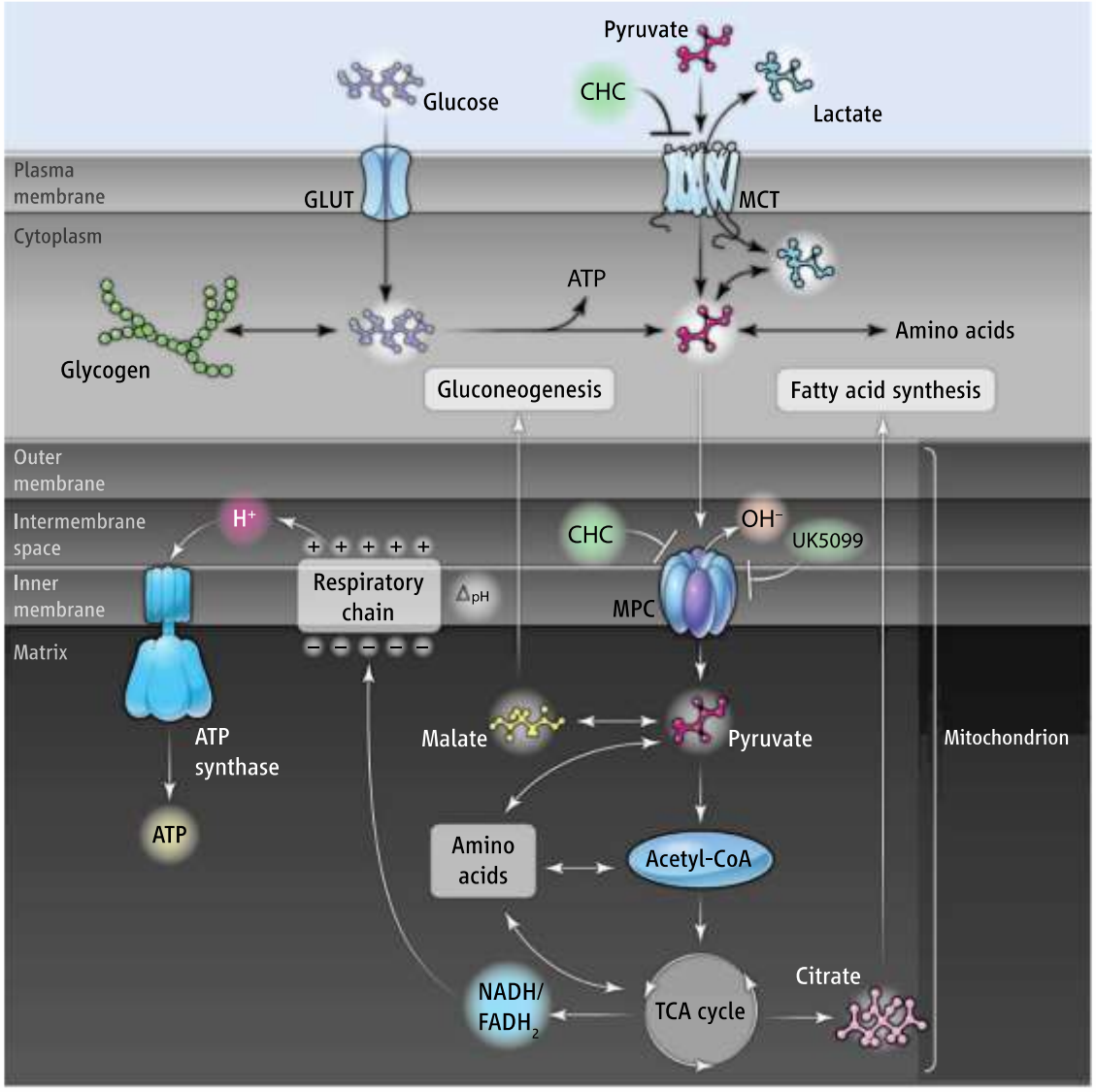
- MPC Knockdown lowers TZD EC_{50}
- UK5099 reproduces plasma membrane glucose uptake and AMPK phosphorylation

  Divakaruni and Murphy (2013) *PNAS*
- ^{125}I-pioglitazone binds to MPC

  Colca and Kletzein (2013) *PLoS ONE*

Image from Divakaruni AS and Murphy AN (2012) *Science*

The mitochondrial pyruvate carrier (MPC)



- MPC Knockdown lowers TZD EC_{50}
- UK5099 reproduces plasma membrane glucose uptake and AMPK phosphorylation

  Divakaruni and Murphy (2013) *PNAS*
- ^{125}I-pioglitazone binds to MPC

  Colca and Kletzein (2013) *PLoS ONE*

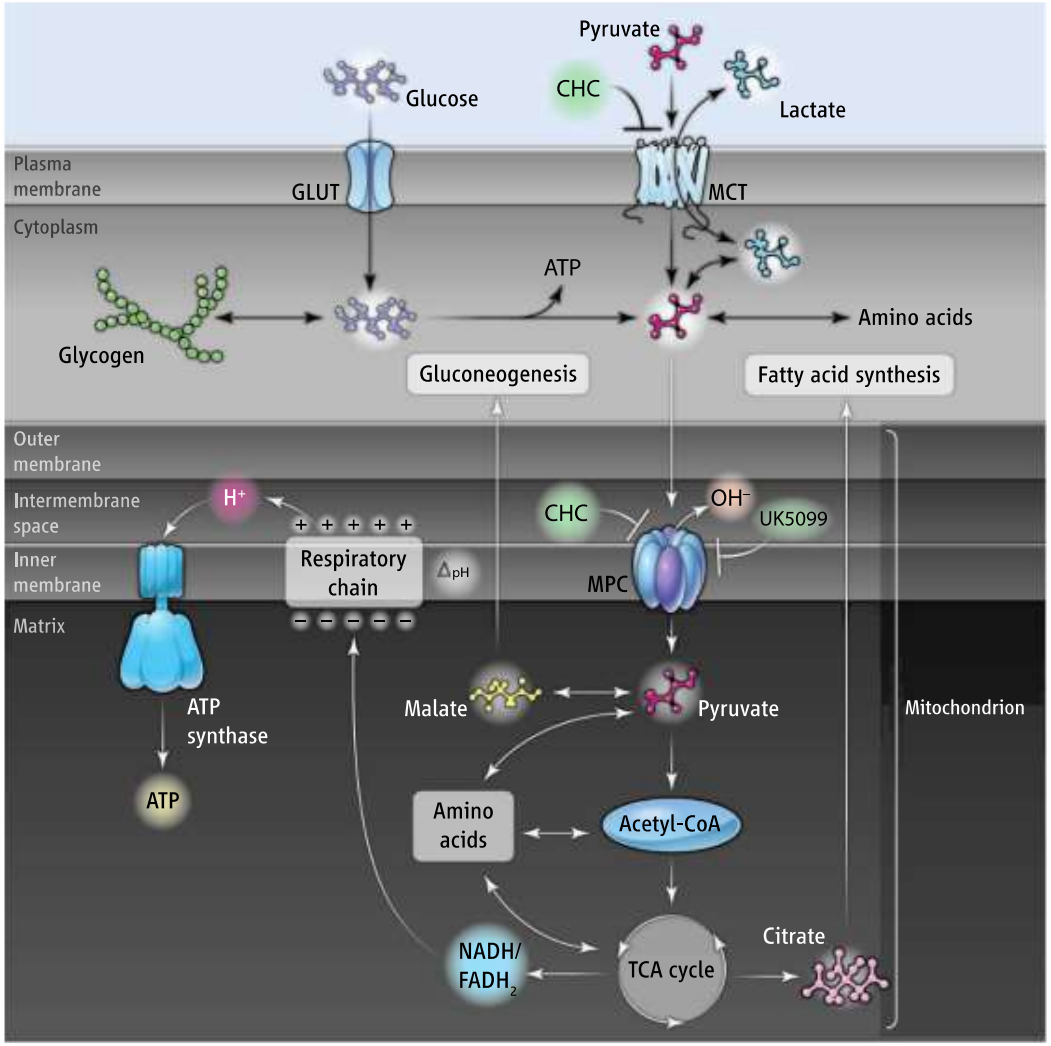
A METABOLOMICS APPROACH?

(1) Metabolite changes are not always direct indications of pathways

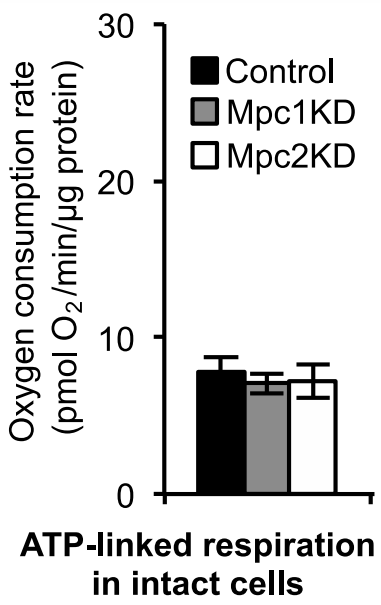
(2) Attributing effects to transcriptional agonism vs. MPC inhibition

Image from Divakaruni AS and Murphy AN (2012) *Science*

Using stable knockdown to understand the cellular response to reduced MPC activity

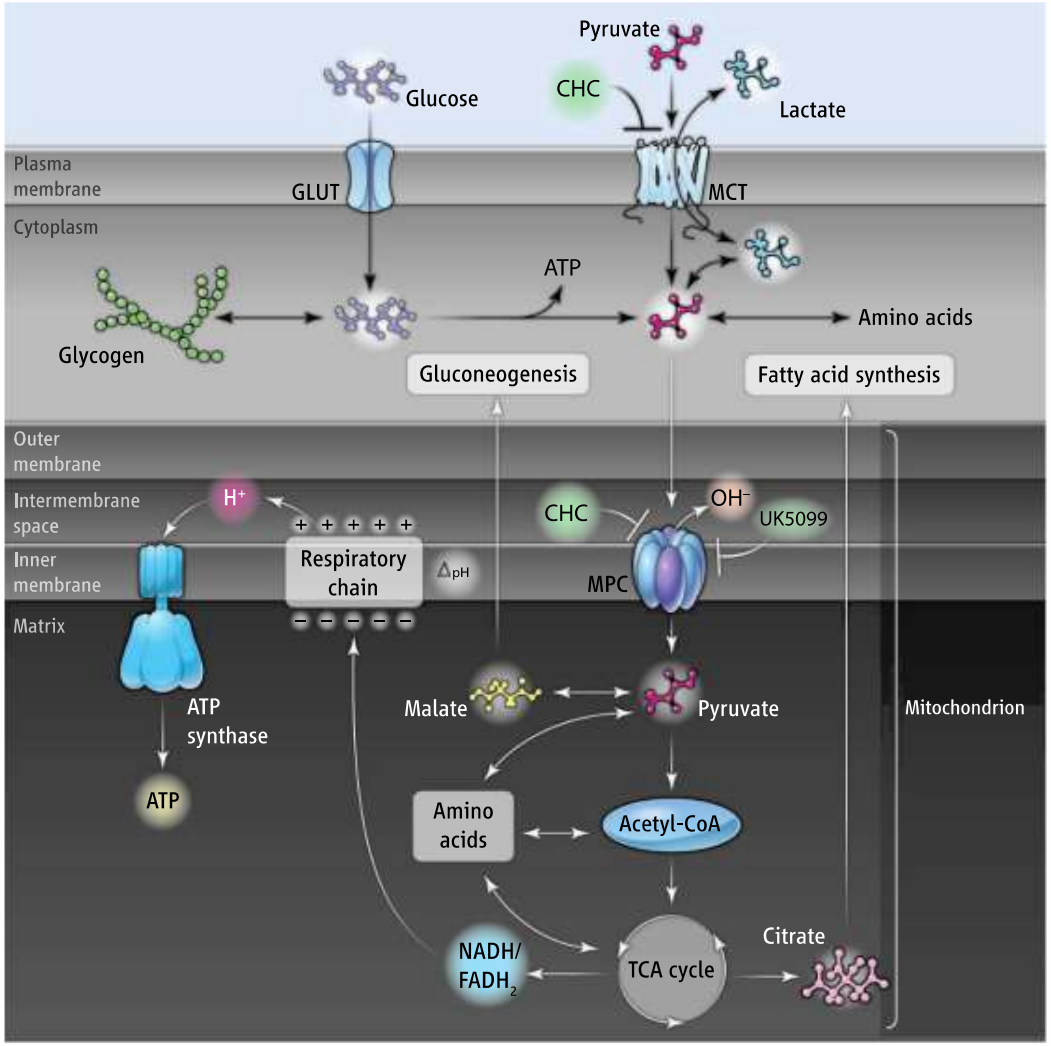


KNOCKING DOWN MPC COMPONENTS IN MYOCYTES DOESN'T ALTER MITOCHONDRIAL ATP PRODUCTION (!)

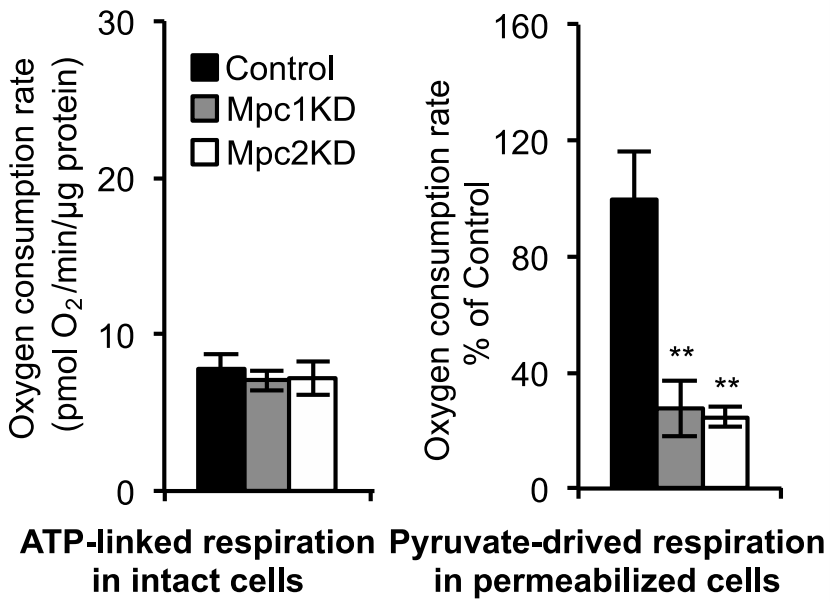


From Vacanti, Divakaruni...and Metallo (2014) *Mol. Cell*

Using stable knockdown to understand the cellular response to reduced MPC activity



KNOCKING DOWN MPC COMPONENTS IN MYOCYTES DOESN'T ALTER MITOCHONDRIAL ATP PRODUCTION (!)



From Vacanti, Divakaruni...and Metallo (2014) *Mol. Cell*

Metabolism, two ways

(1) MEASURE RATES OF INPUTS AND OUTPUTS

(2) TAKE A DETAILED SNAPSHOT IN TIME

(Metabolomics, stable isotope tracing)

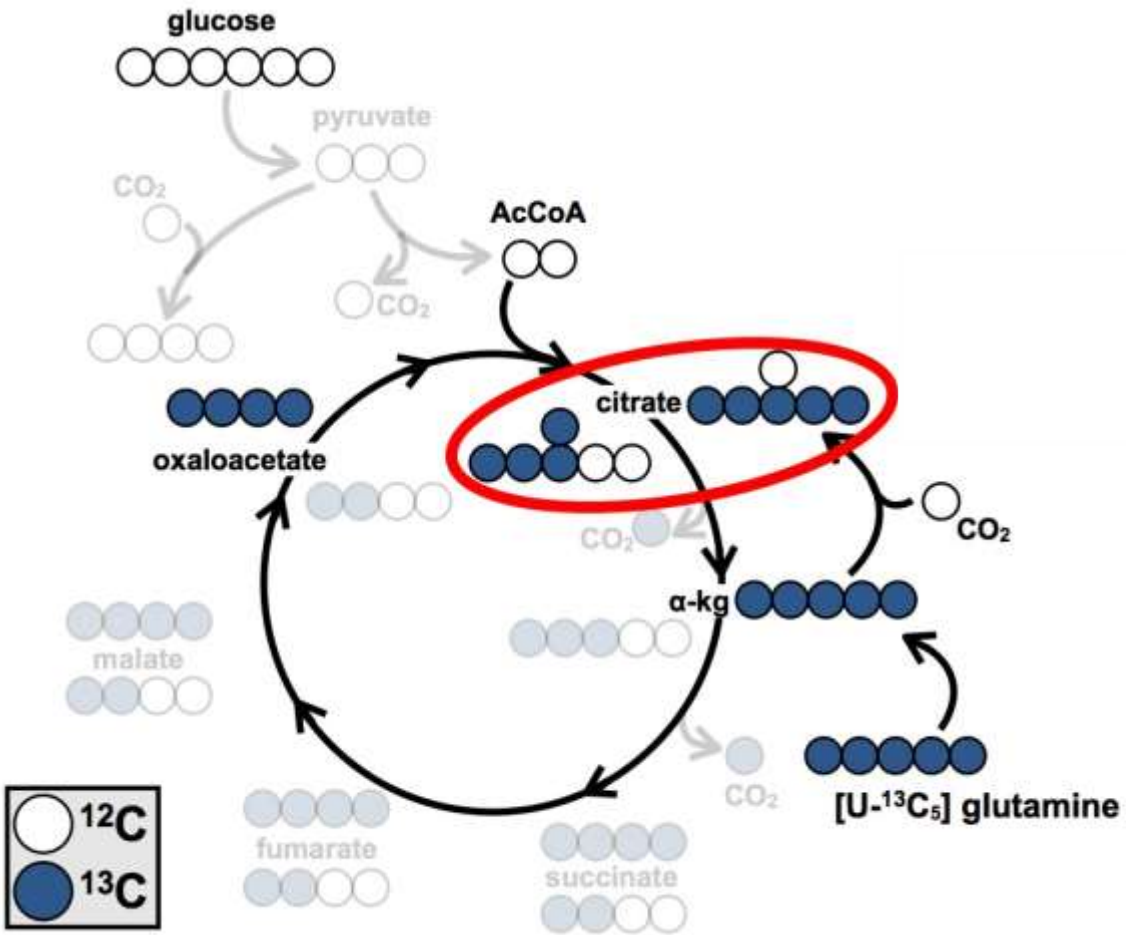
Granular detail from metabolite abundances

Relative enrichment of intermediates from labeled substrates

Combining respiration and stable isotope tracing to fully reveal cell metabolism

Isotopically labeled substrates (i.e. glucose, glutamine, etc.) are offered as tracers

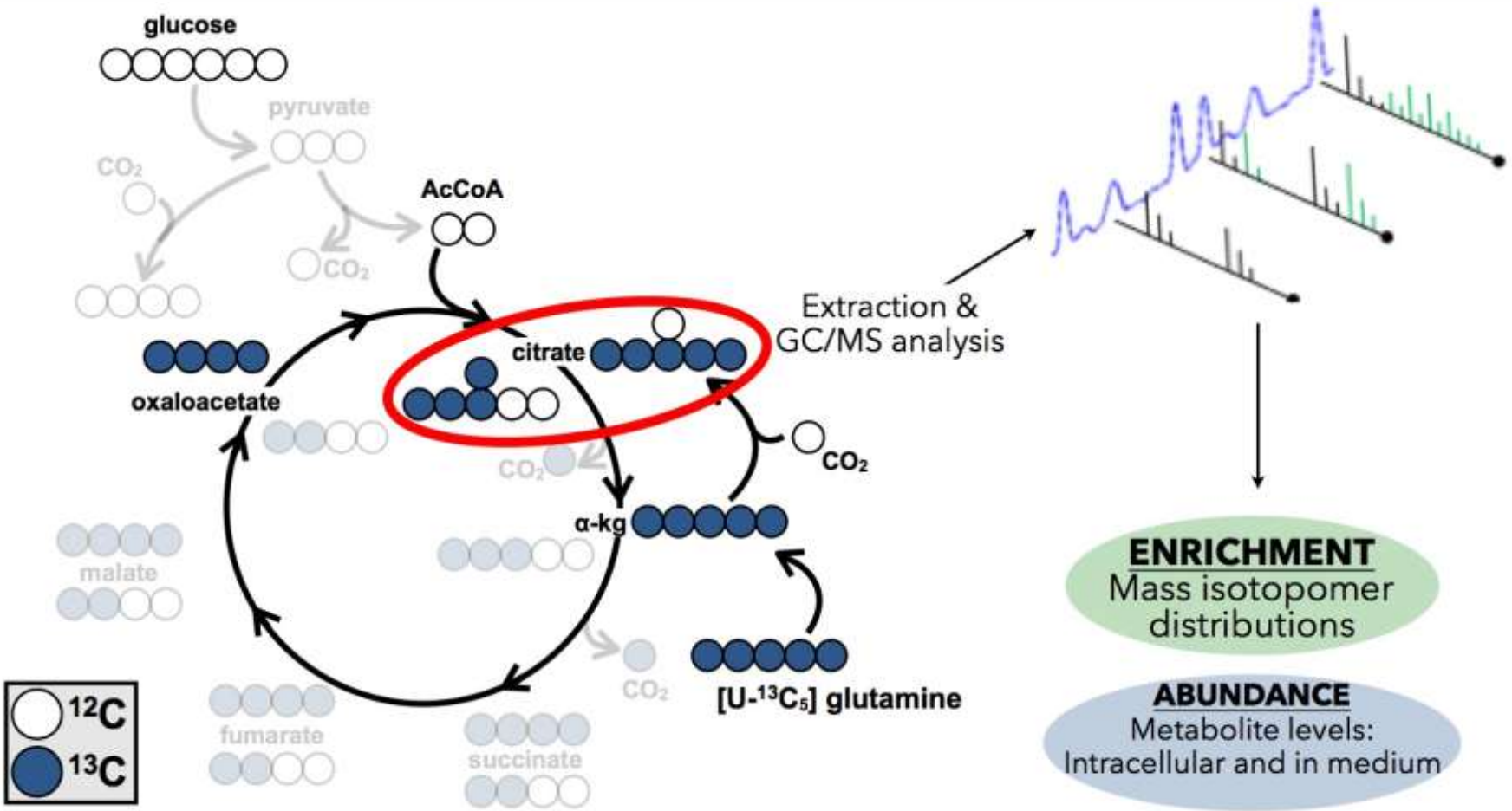
^{13}C labeling of metabolite pools can map the pattern of carbon flux



Combining respiration and stable isotope tracing to fully reveal cell metabolism

Isotopically labeled substrates (i.e. glucose, glutamine, etc.) are offered as tracers

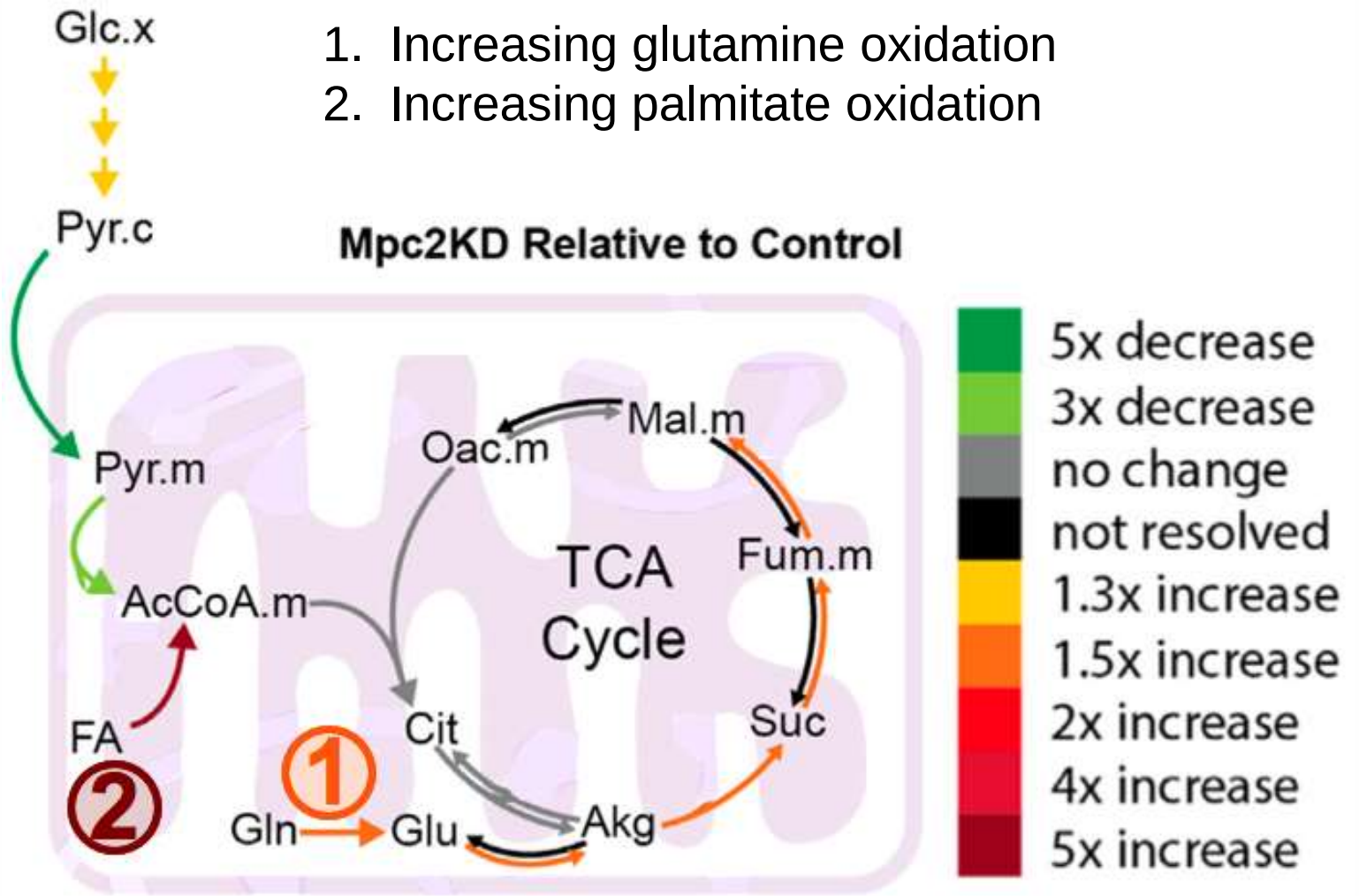
^{13}C labeling of metabolite pools can map the pattern of carbon flux



The MPC is a switch that can potentiate the oxidation of amino acids and fatty acids

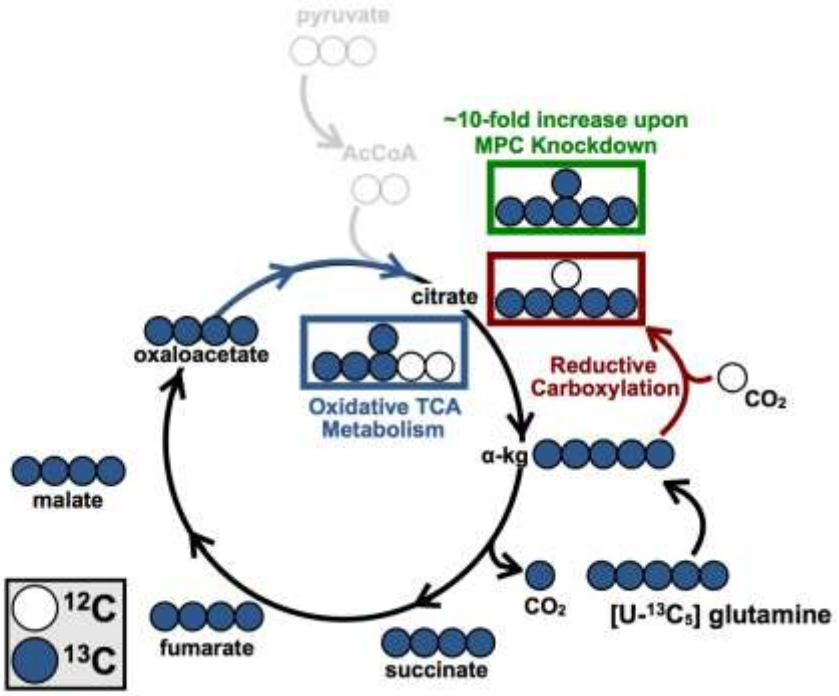
MYOCYTES ADAPT TO DECREASED MITOCHONDRIAL PYRUVATE UPTAKE BY:

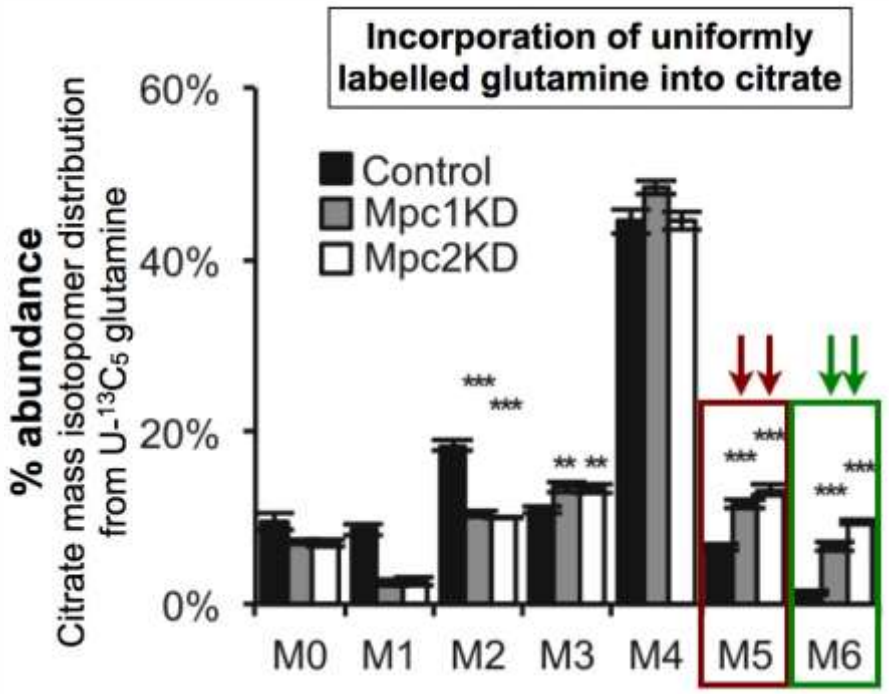
1. Increasing glutamine oxidation
2. Increasing palmitate oxidation



Stable isotope tracing can reveal information that respirometry cannot

For research use only. Not for use in diagnostic procedures.





Malic enzyme activity increases upon MPC knockdown

Glutamine Oxidation Maintains the TCA Cycle and Cell Survival during Impaired Mitochondrial Pyruvate Transport

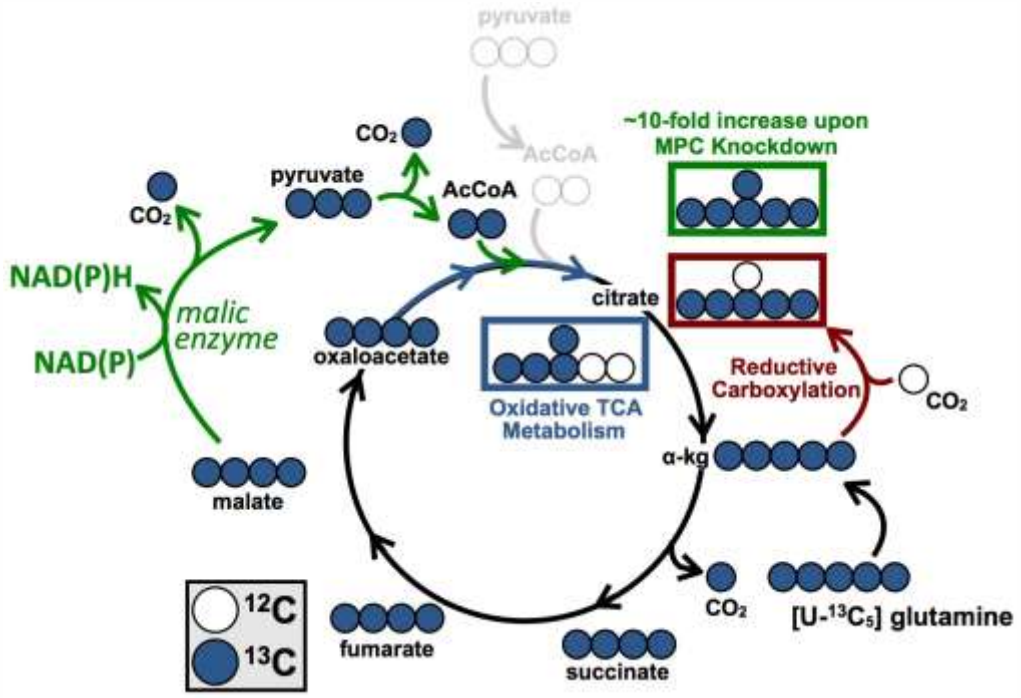
Chendong Yang,[1] Bookyung Ko,[1] Christopher T. Hensley,[1] Lei Jiang,[1] Ajla T. Wasti,[1,2] Jiyeon Kim,[1] Jessica Sudderth,[1] Maria Antonietta Calvaruso,[1] Lloyd Lumata,[3] Matthew Mitsche,[4] Jared Rutter,[5] Matthew E. Merritt,[3] and Ralph J. DeBerardinis[1,2,4,*]

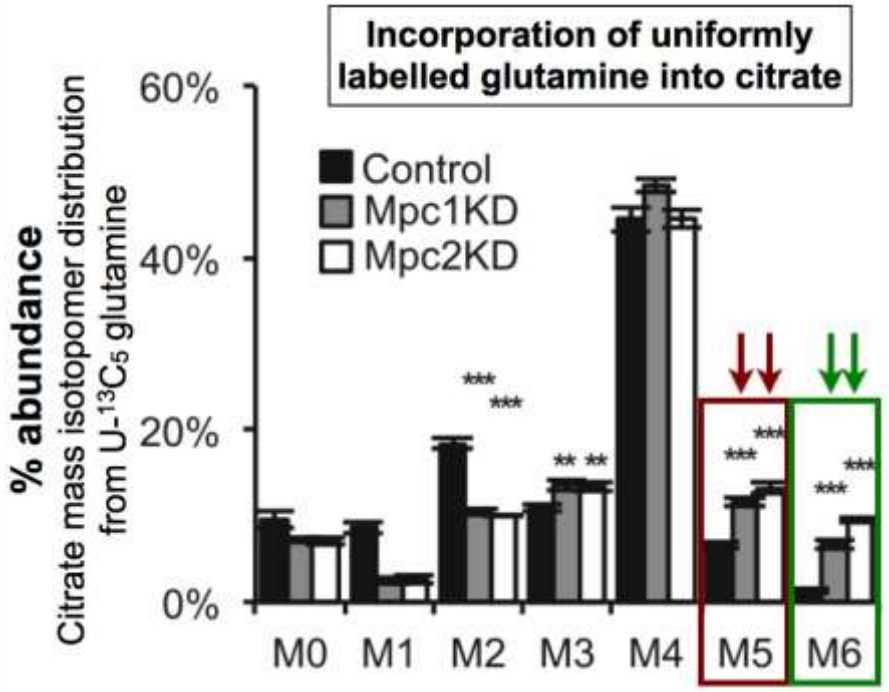
Molecular Cell *56* November 6, 2014

Regulation of Substrate Utilization by the Mitochondrial Pyruvate Carrier

Nathaniel M. Vacanti,[1] Ajit S. Divakaruni,[2] Courtney R. Green,[1] Seth J. Parker,[1] Robert R. Henry,[3,4] Theodore P. Ciaraldi,[3,4] Anne N. Murphy,[2] and Christian M. Metallo[1,*]

Molecular Cell *56* November 6, 2014

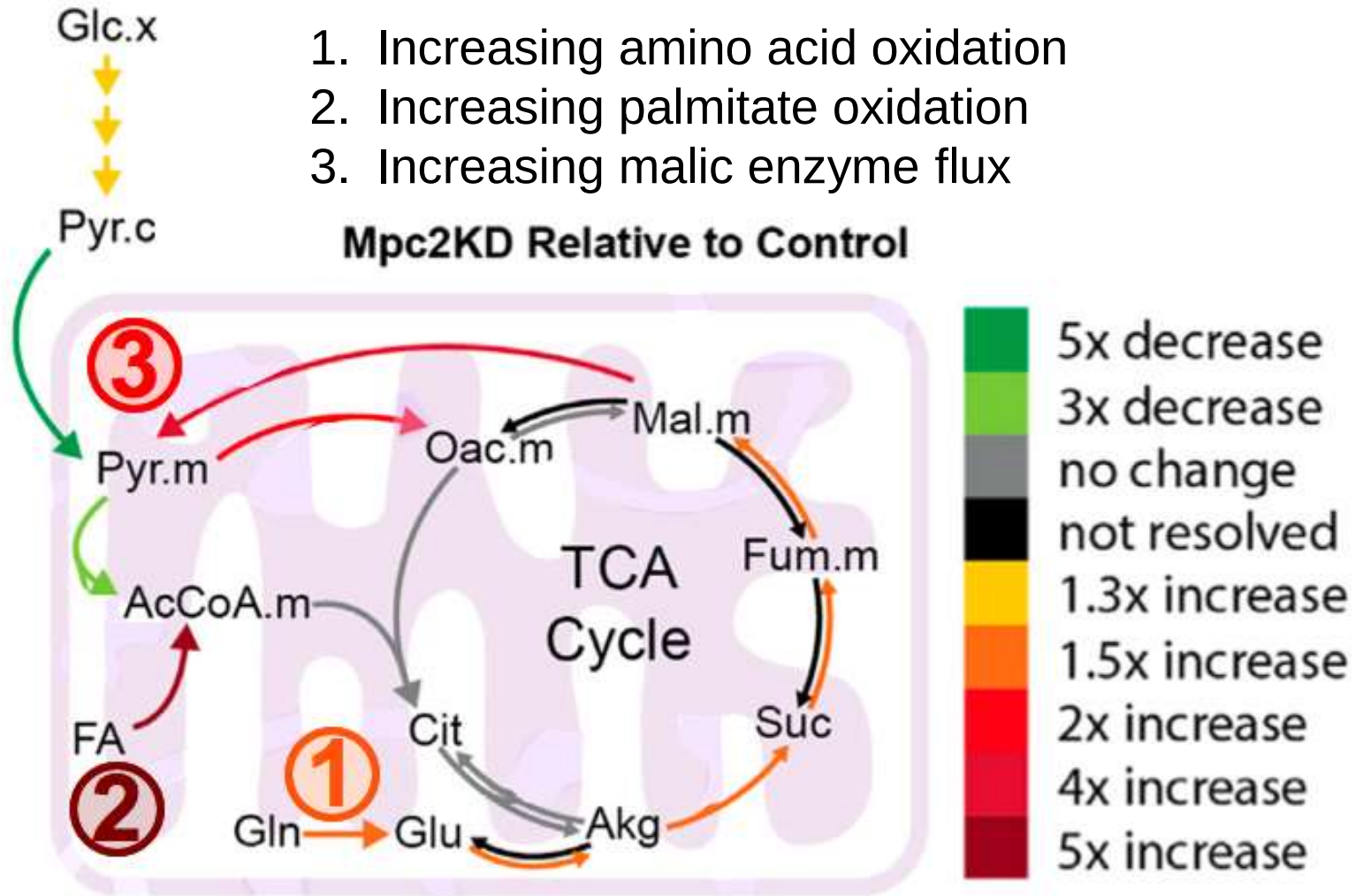




The MPC is a “switch” that can potentiate metabolic flexibility

MYOCYTES ADAPT TO DECREASED MITOCHONDRIAL PYRUVATE UPTAKE BY:

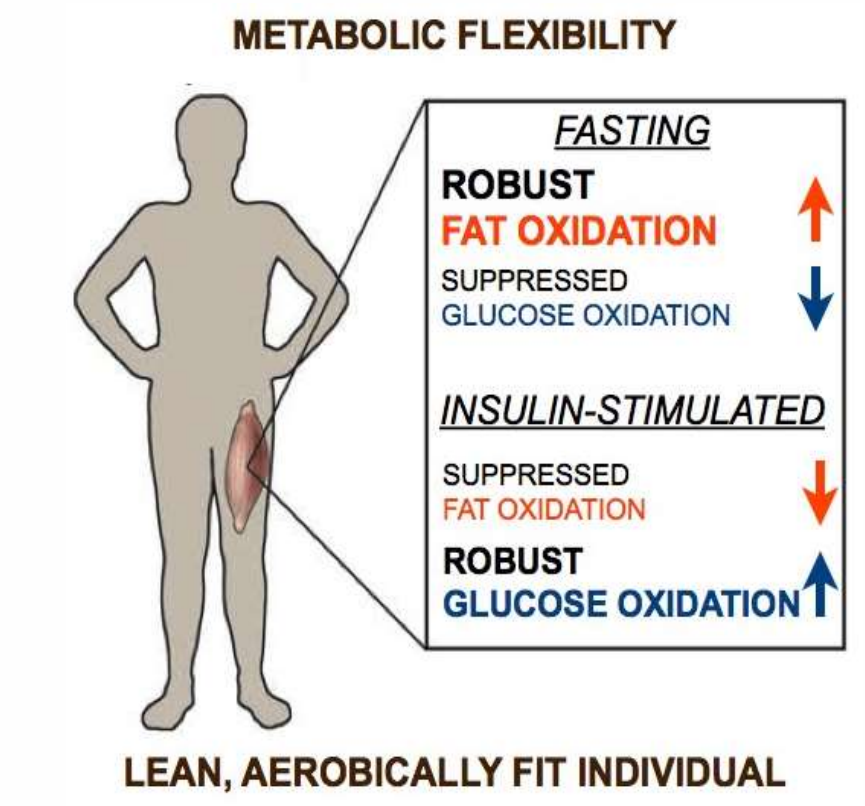
1. Increasing amino acid oxidation
2. Increasing palmitate oxidation
3. Increasing malic enzyme flux

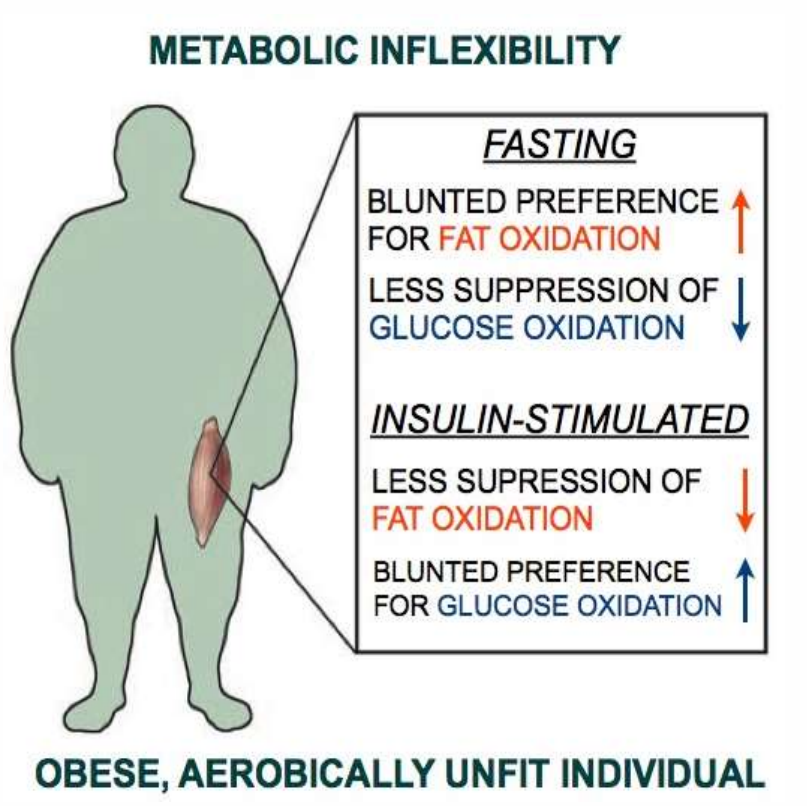


Adapted from Vacanti, Divakaruni...and Metallo (2014) *Mol. Cell*

The MPC is a “switch” that can potentiate metabolic flexibility

Metabolic flexibility is a characteristic of healthy skeletal muscle



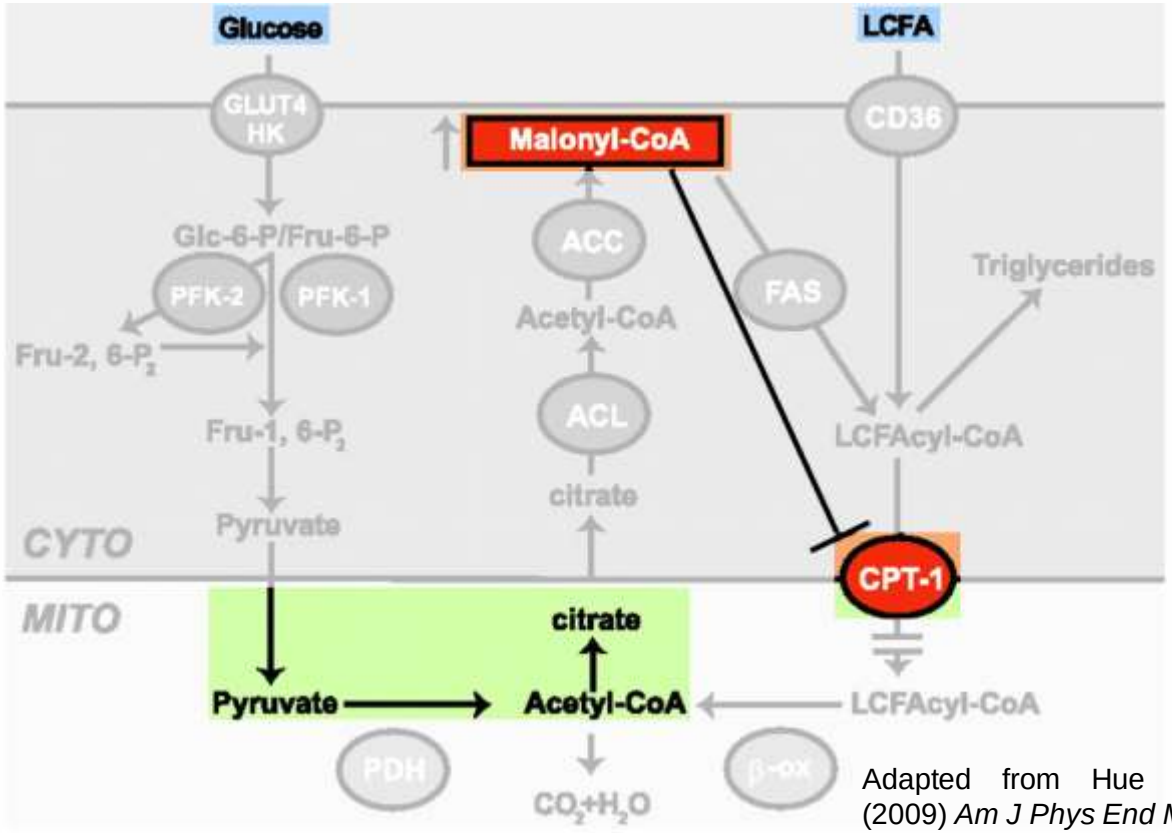


Adapted from Kelley DE (2005) *JCI*

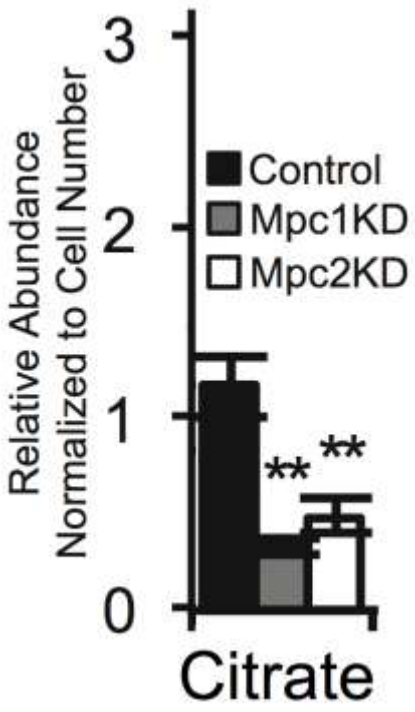
The MPC is a “switch” that can potentiate metabolic flexibility

MPC inhibition potentiates fatty acid oxidation (by reducing malonyl CoA?)

An inability to increase lipid oxidation when faced with high supply can cause increased muscle fat accumulation and insulin resistance



Adapted from Hue and Taegtmeyer (2009) *Am J Phys End Metab*



From Vacanti,...and Metallo (2014) *Mol. Cell*

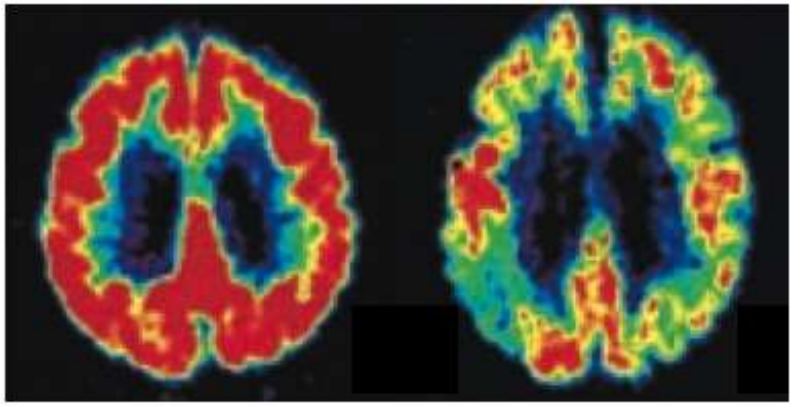
Is there a metabolic component to neurodegenerative disease?

TYPE 2 DIABETES IS A STRONG RISK FACTOR FOR AD

SHARED PATHOLOGY BETWEEN T2D AND AD

- Insulin resistance
- Amyloidosis
- Oxidative stress
- Cognitive impairment
- Neural tissue atrophy

Healthy brain Mild-to-moderate AD



From Small GW…and Phelps ME (2000) *PNAS*

Is there a metabolic component to neurodegenerative disease?

TYPE 2 DIABETES IS A STRONG RISK FACTOR FOR AD

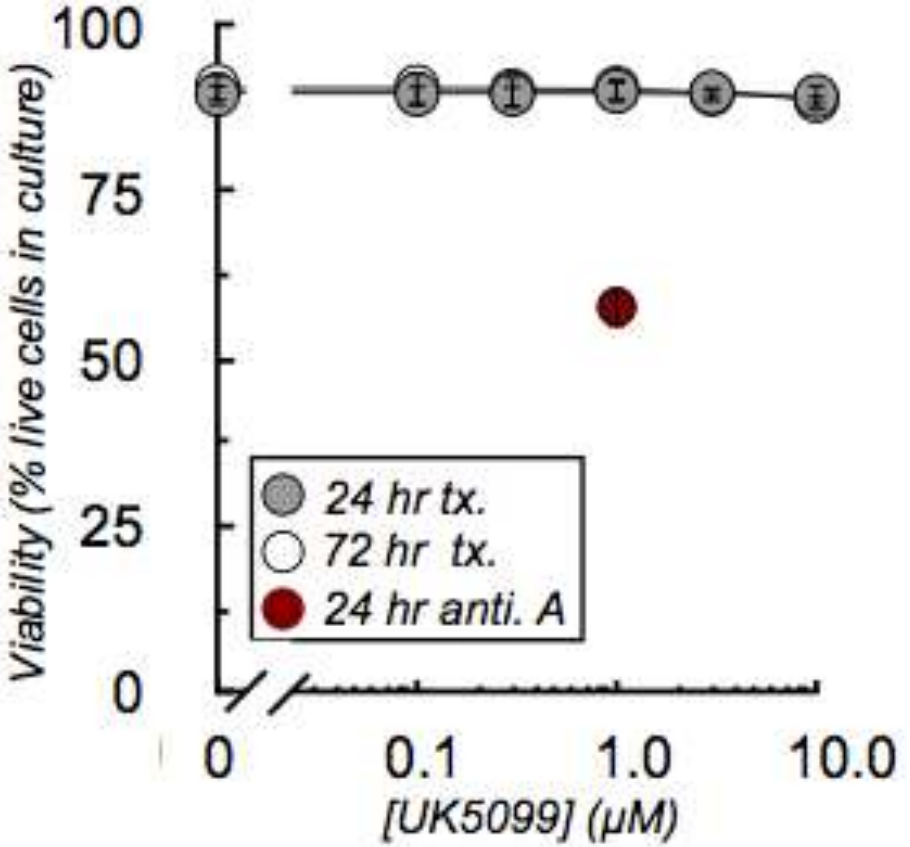
SHARED PATHOLOGY BETWEEN T2D AND AD

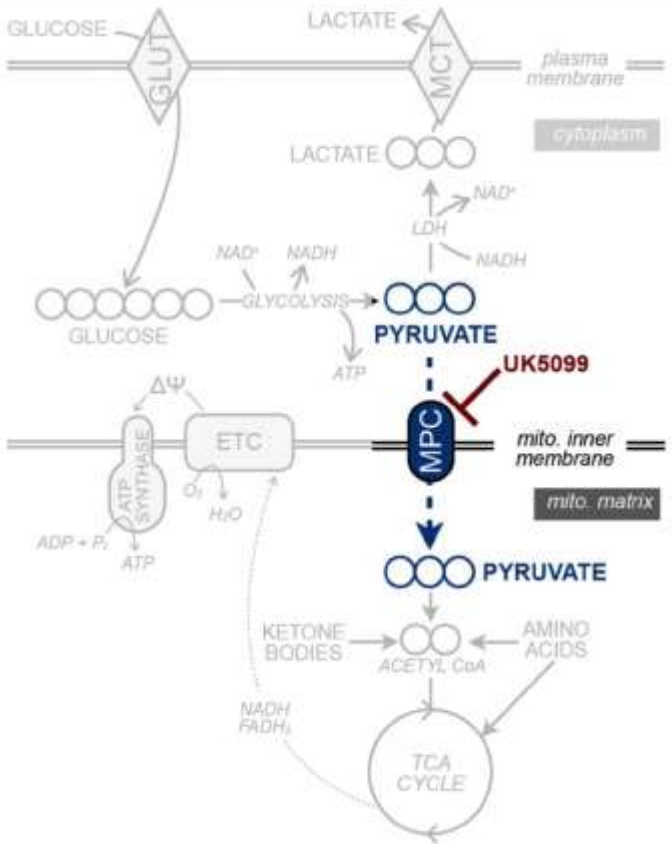
- Insulin resistance
- Amyloidosis
- Oxidative stress
- Cognitive impairment
- Neural tissue atrophy

REPURPOSING OF ANTI-DIABETIC THERAPIES FOR AD

- Intranasal insulin delivery
- Incretin analogs
- Thiazolidinediones (TZDs)

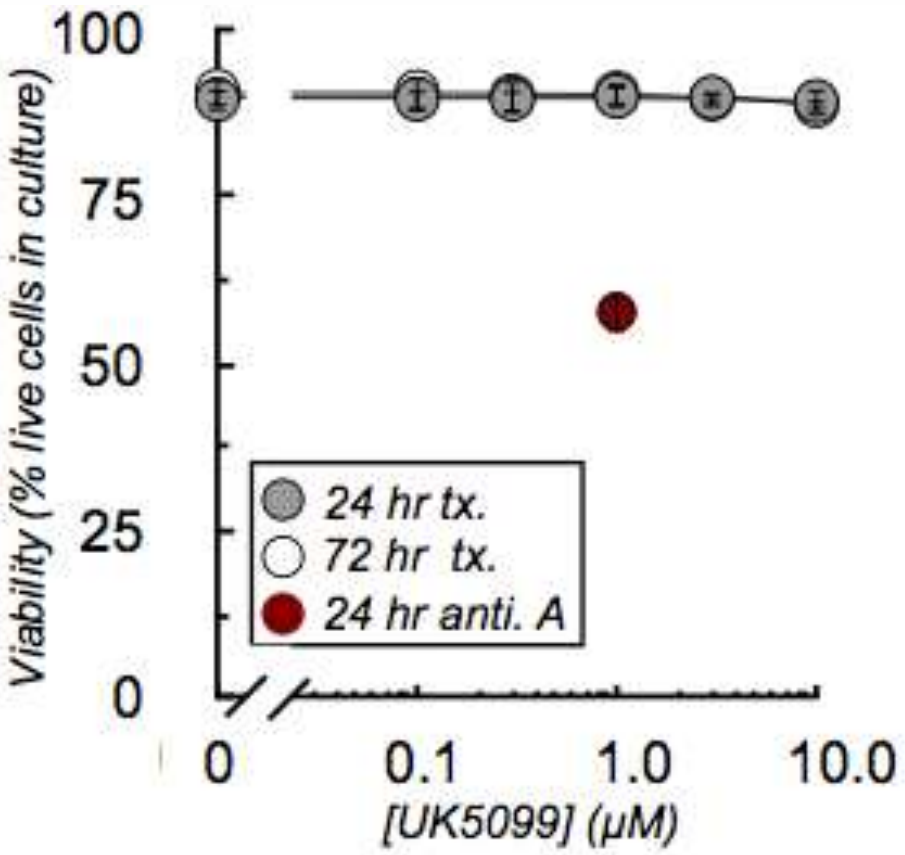
Viability is unchanged despite reduced MPC activity

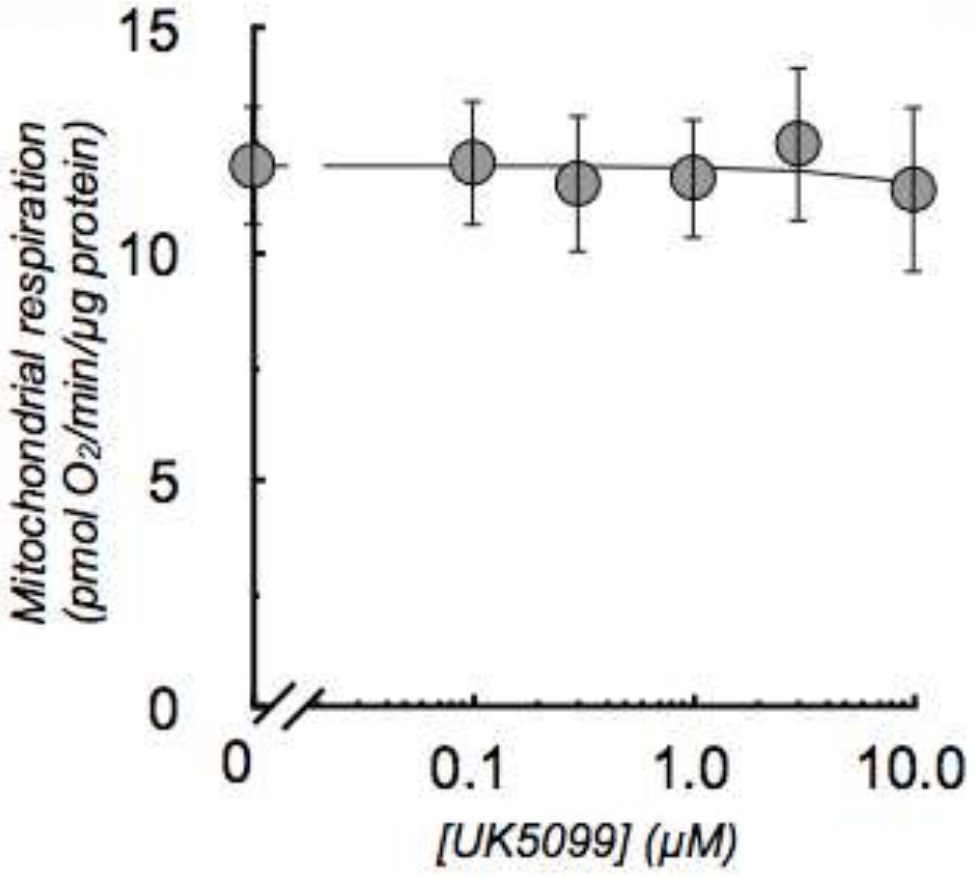




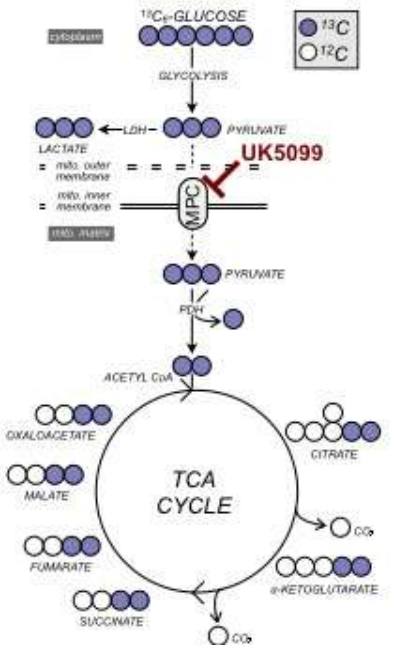
Divakaruni…and Murphy (2017) J. Cell Biol.

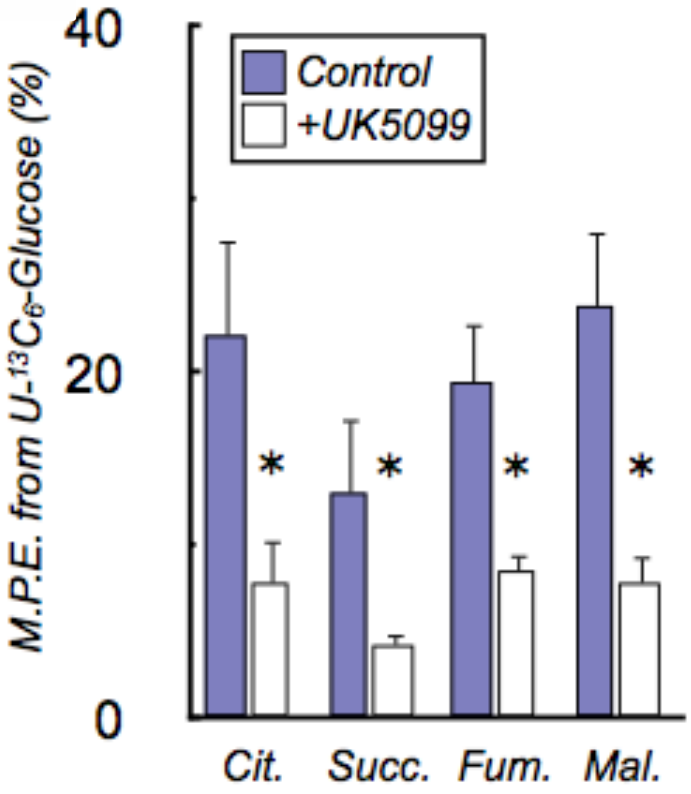
Global bioenergetics unchanged despite reduced MPC activity

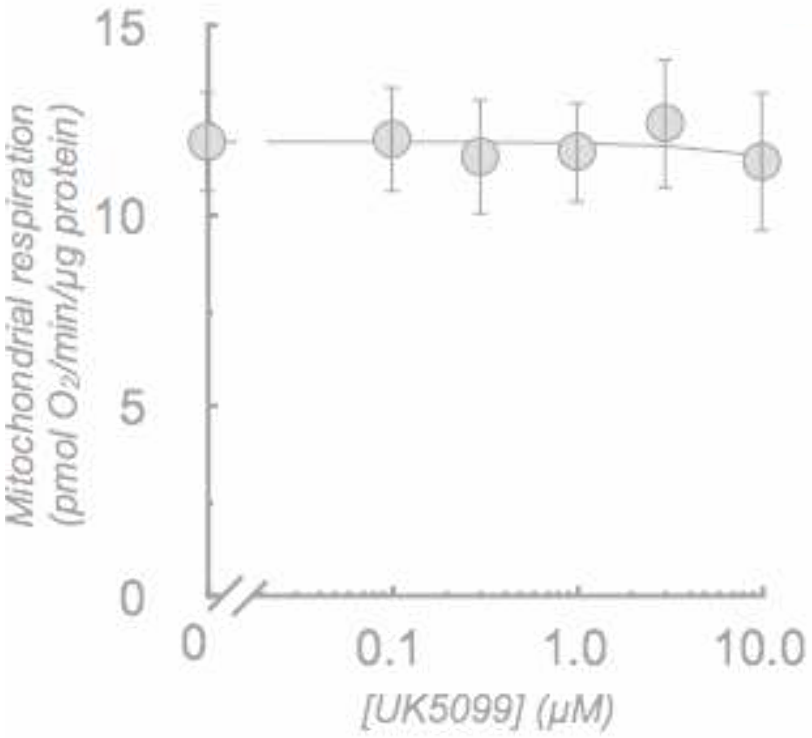




Global bioenergetics unchanged despite reduced MPC activity

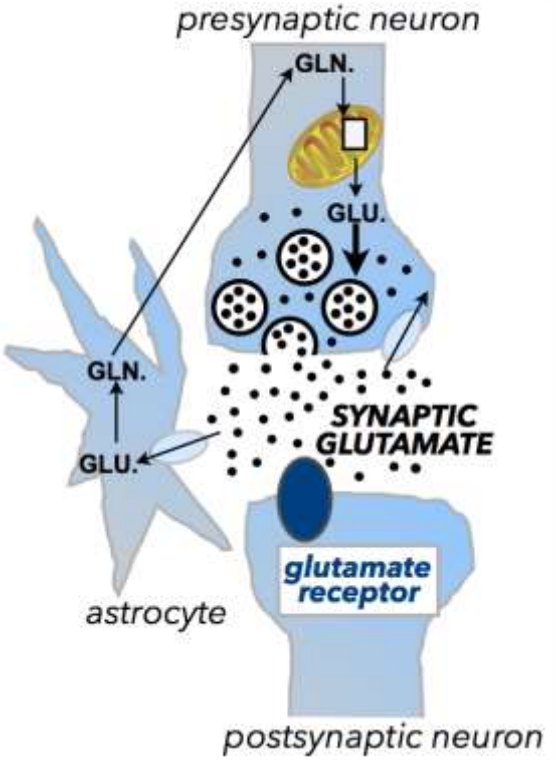






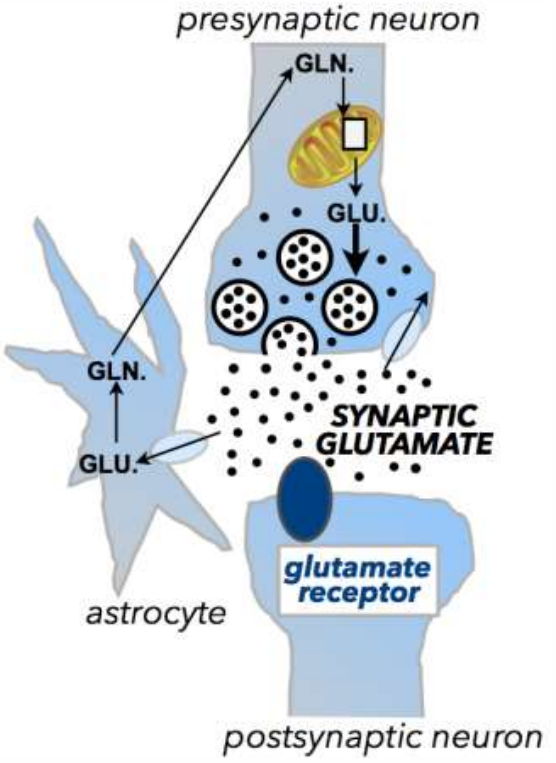
Challenging neurons by stimulating glutamate receptors

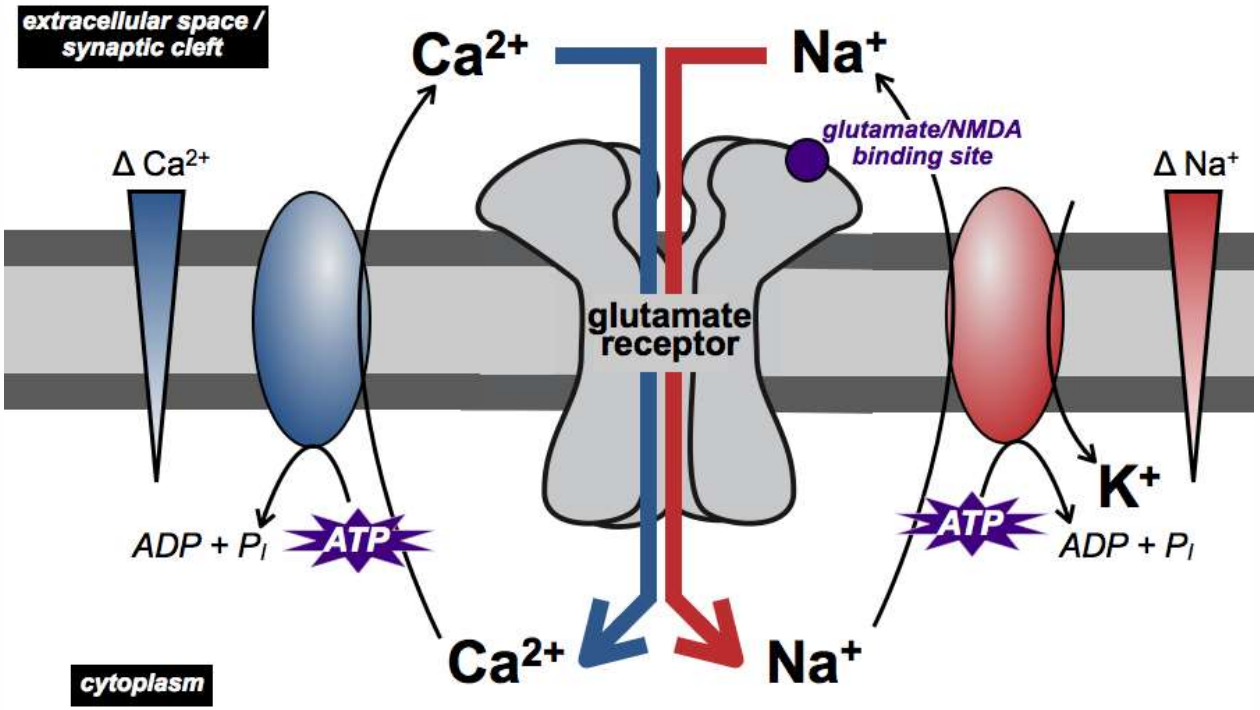
Glutamate is the dominant excitatory neurotransmitter in the brain (balanced by inhibitory GABA)



Challenging neurons by stimulating glutamate receptors

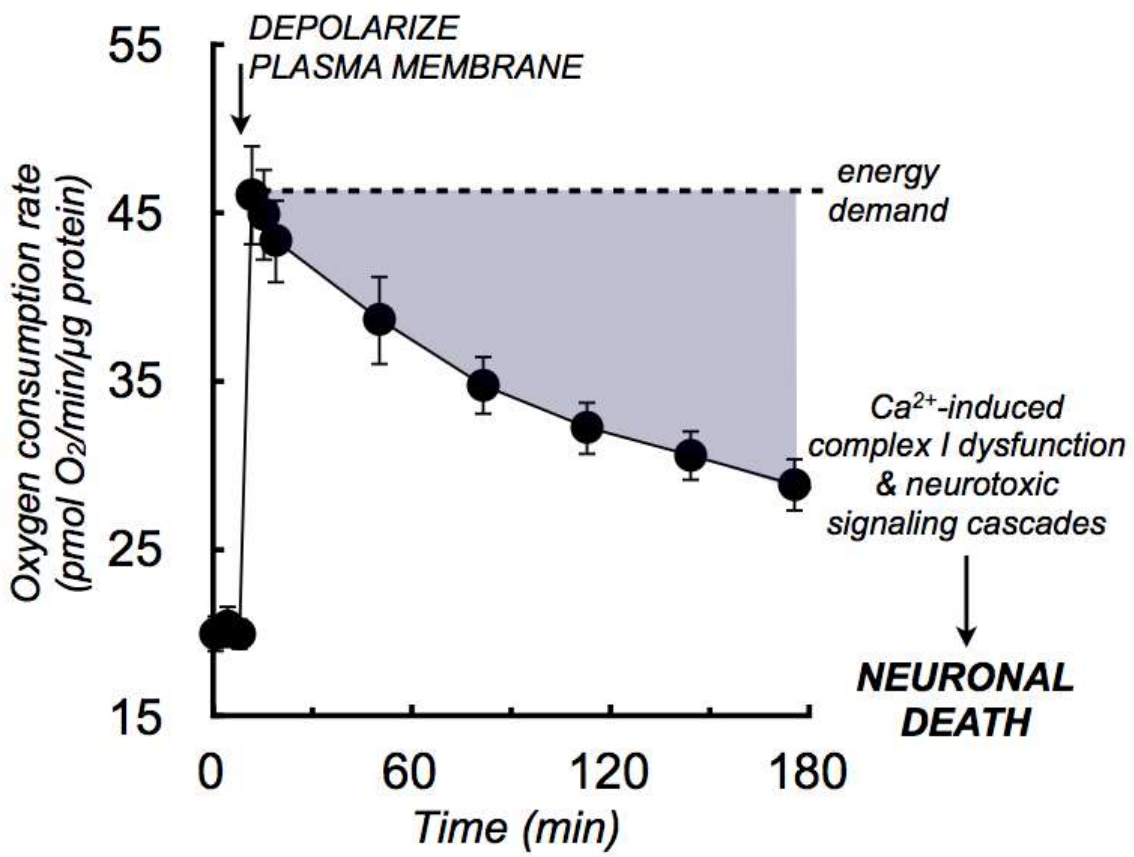
Glutamatergic neurotransmission comes at a tremendous energetic cost

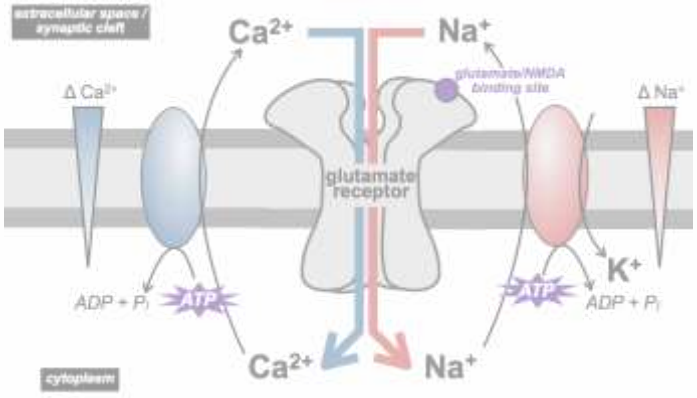




Challenging neurons by stimulating glutamate receptors

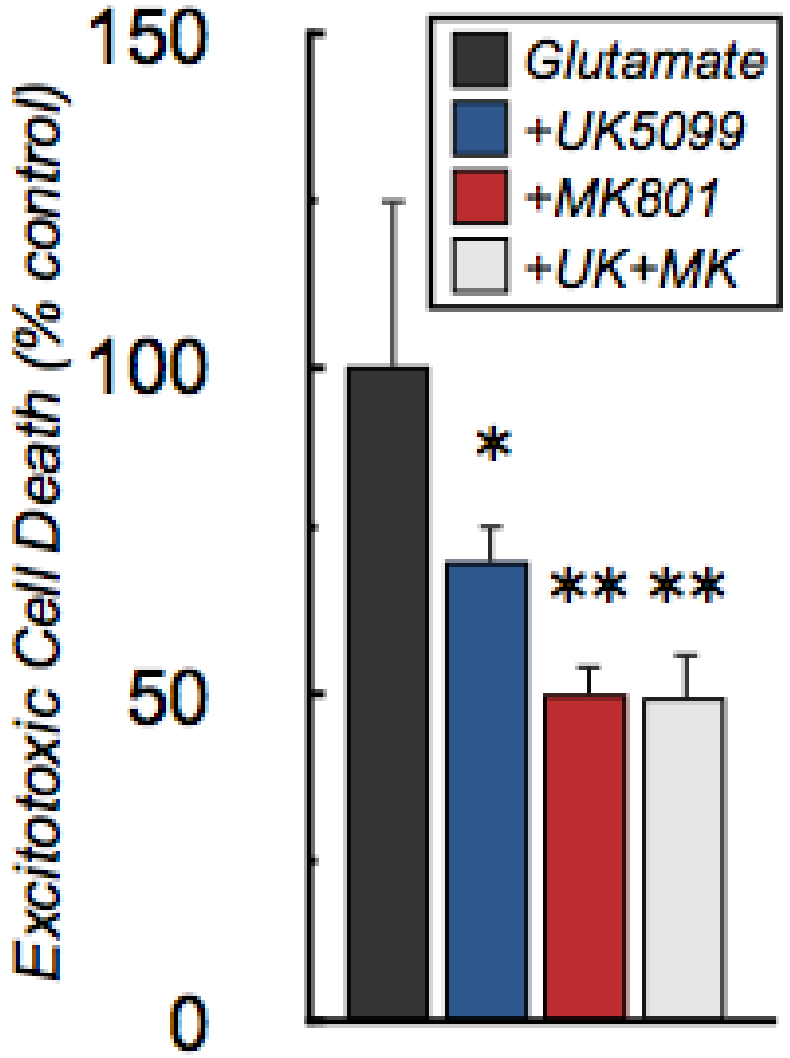
Under conditions of energetic stress or de-energization (stroke, epilepsy, some chronic neurodegeneration), extracellular glutamate rises to toxic levels





MPC inhibition protects from excitotoxic cell death (!)

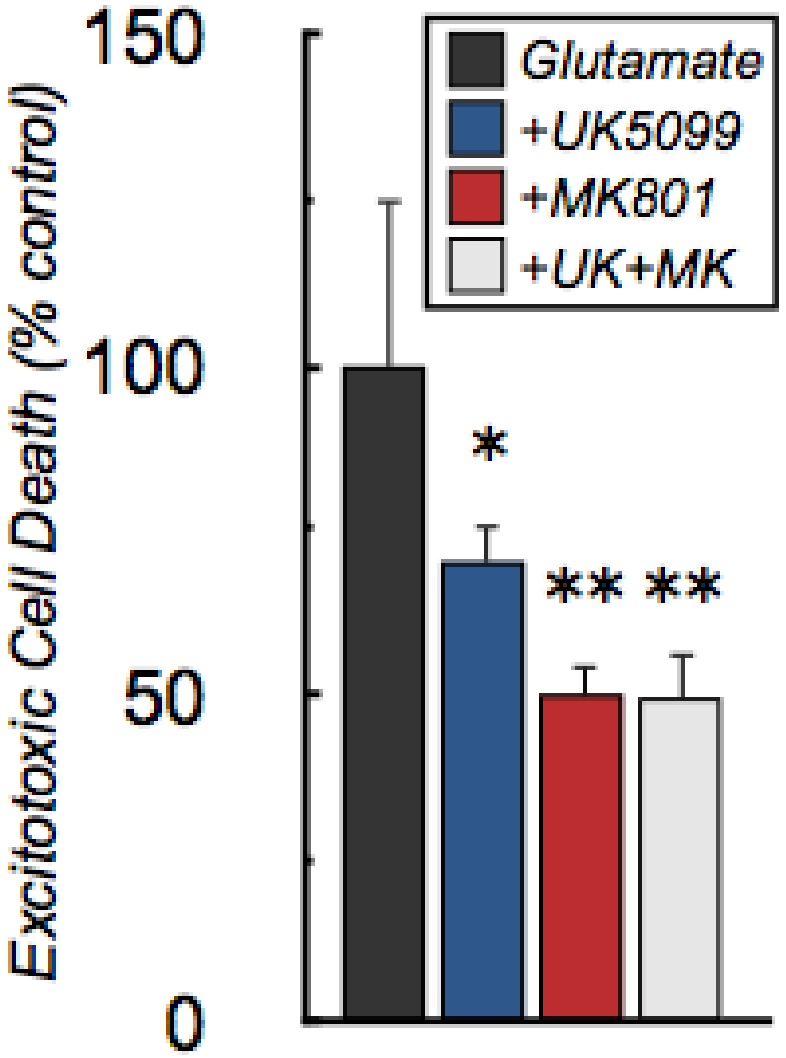
In rich culture medium, inhibiting mitochondrial pyruvate metabolism in neurons protects from excitotoxic neuronal death



MPC inhibition protects from excitotoxic cell death (!)

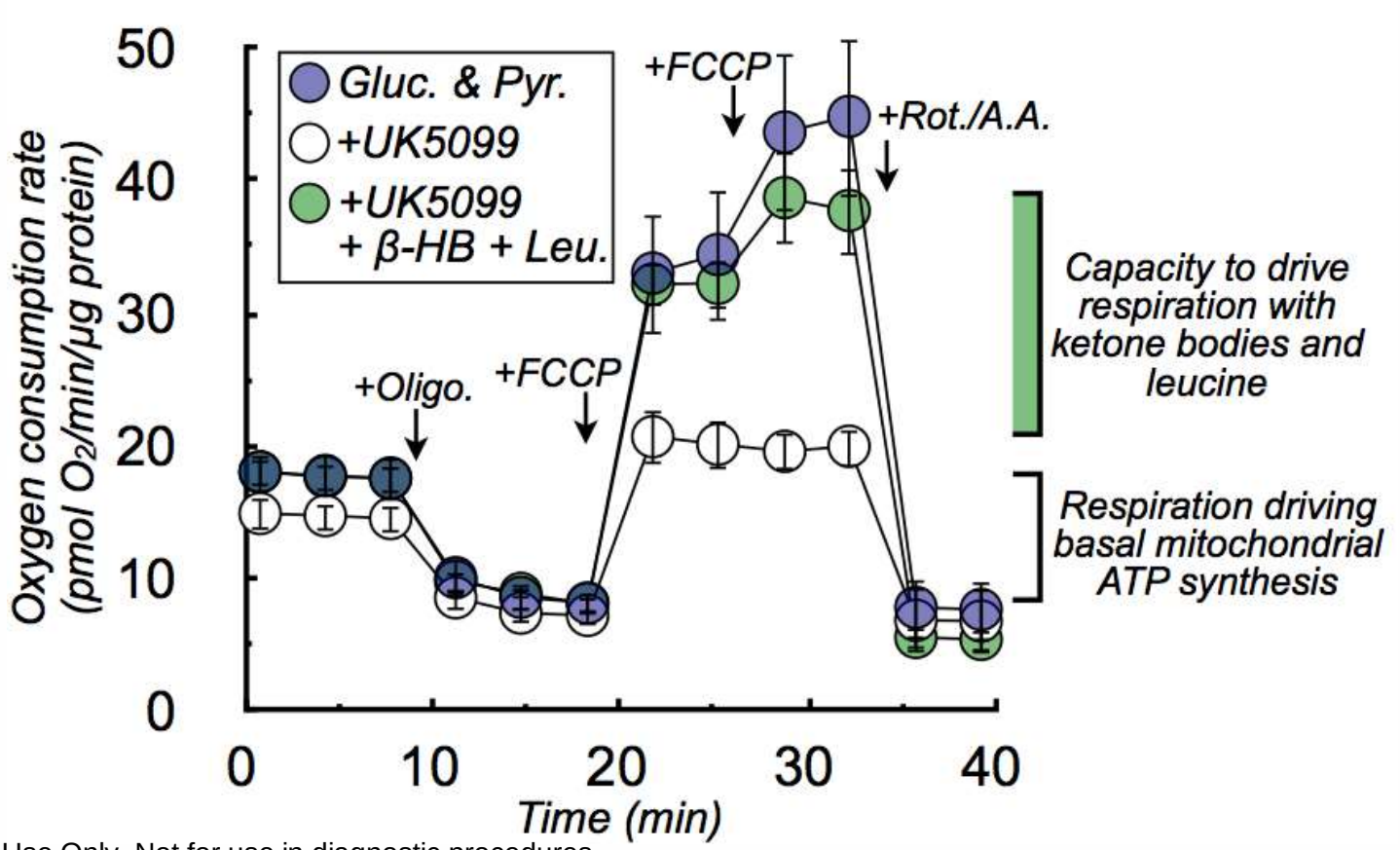
In rich culture medium, inhibiting mitochondrial pyruvate metabolism in neurons protects from excitotoxic neuronal death

How has MPC inhibition rewired neuronal metabolism in a way that is neuroprotective?

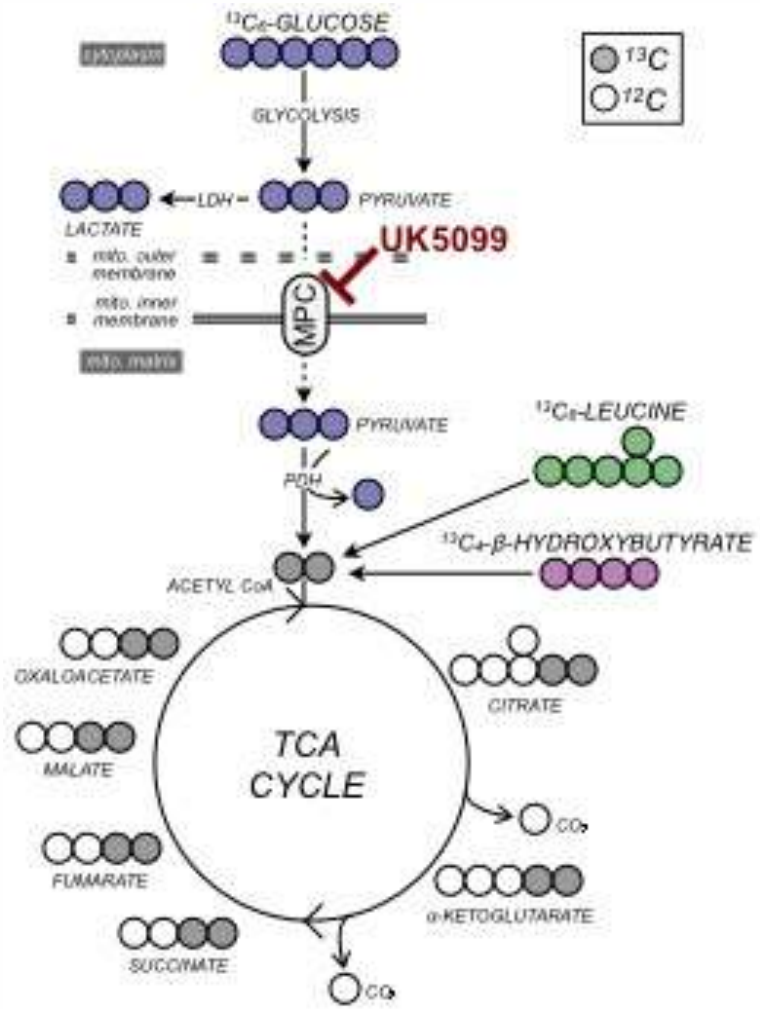


Bioenergetic analysis shows neurons have a huge capacity to oxidize ketone bodies and BCAAs…

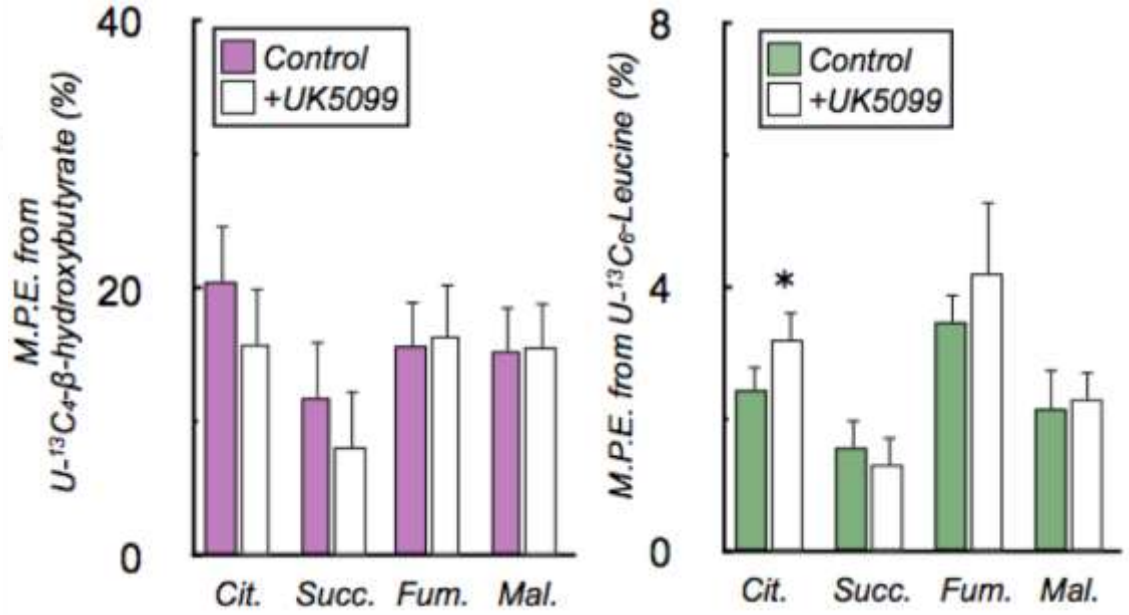
Acute challenge in simple salts medium where substrate preference is assumed *a priori* by the experimenter



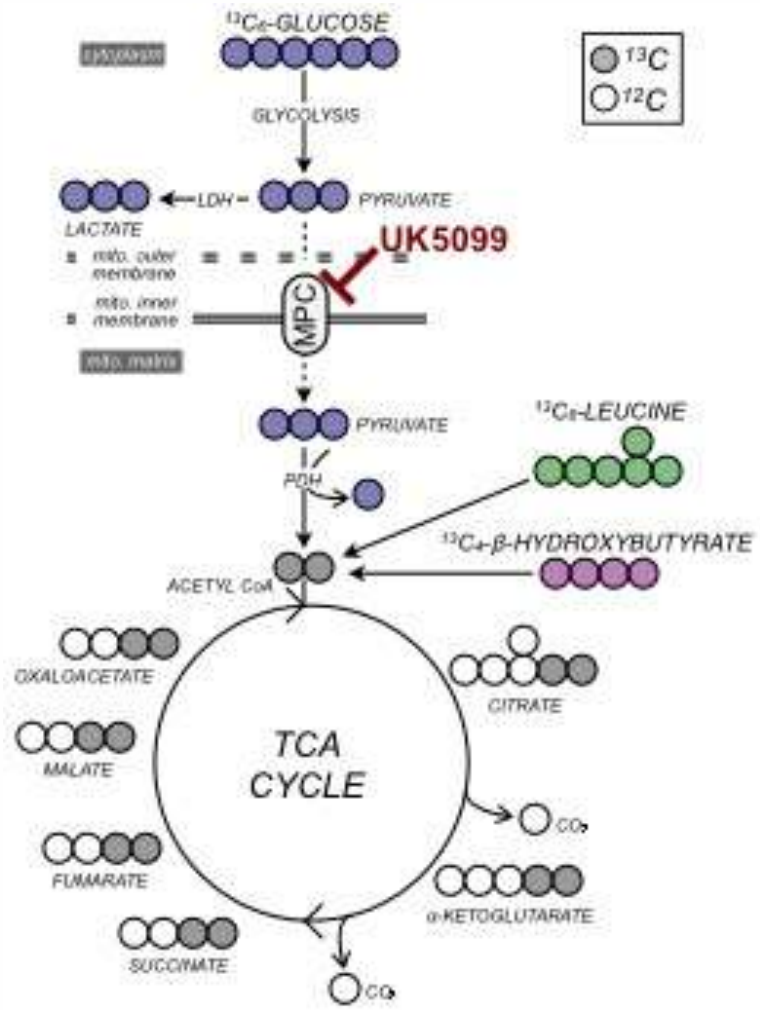
…but stable isotope tracing shows no increase in oxidation of these substrates in response to UK5099



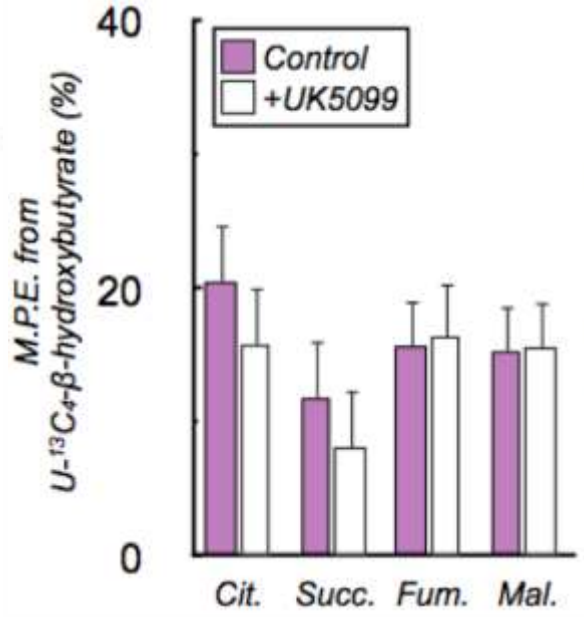
In rich culture medium we see no increase in TCA enrichment from ketone bodies (left, pink) or leucine (right, green) upon MPC inhibition over 24 hr.

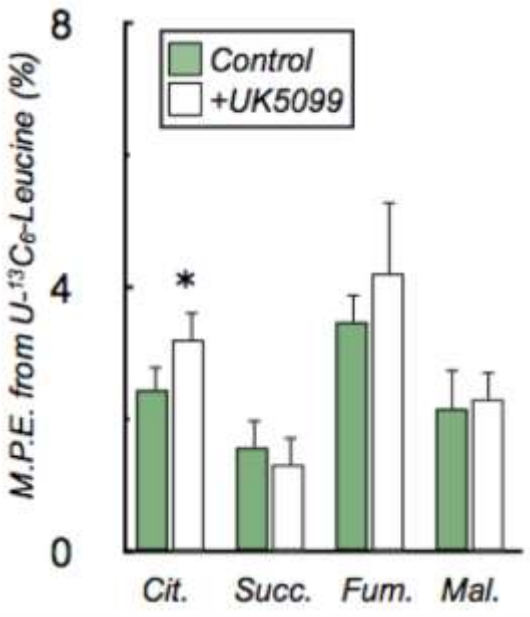


…but stable isotope tracing shows no increase in oxidation of these substrates in response to UK5099



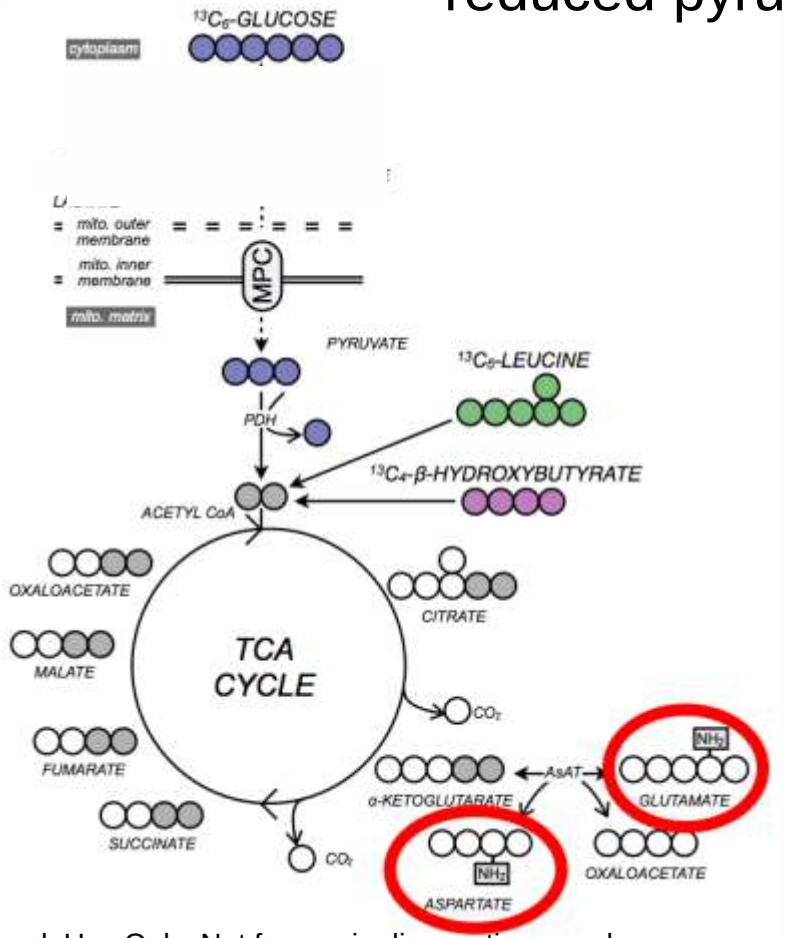
WE MISSED SOMETHING

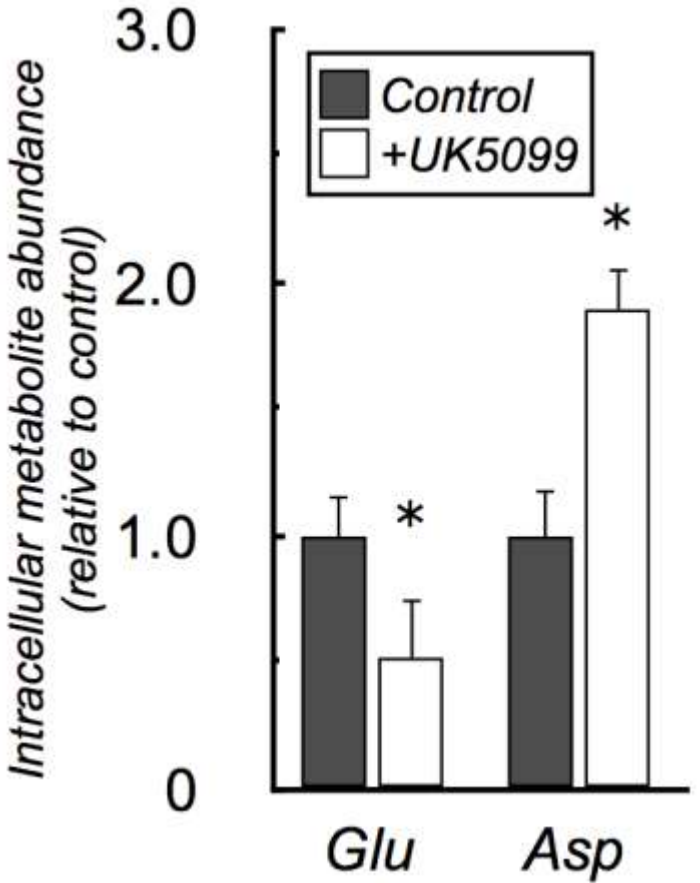




Searching for the protective mechanism of UK5099

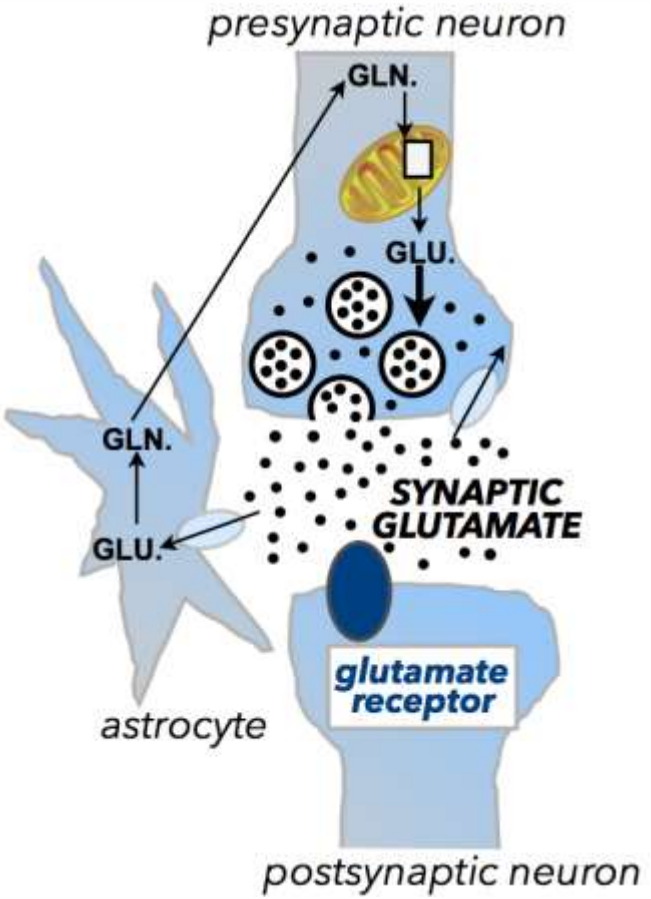
Amino acid levels suggest increased neuronal glutamate oxidation upon reduced pyruvate uptake

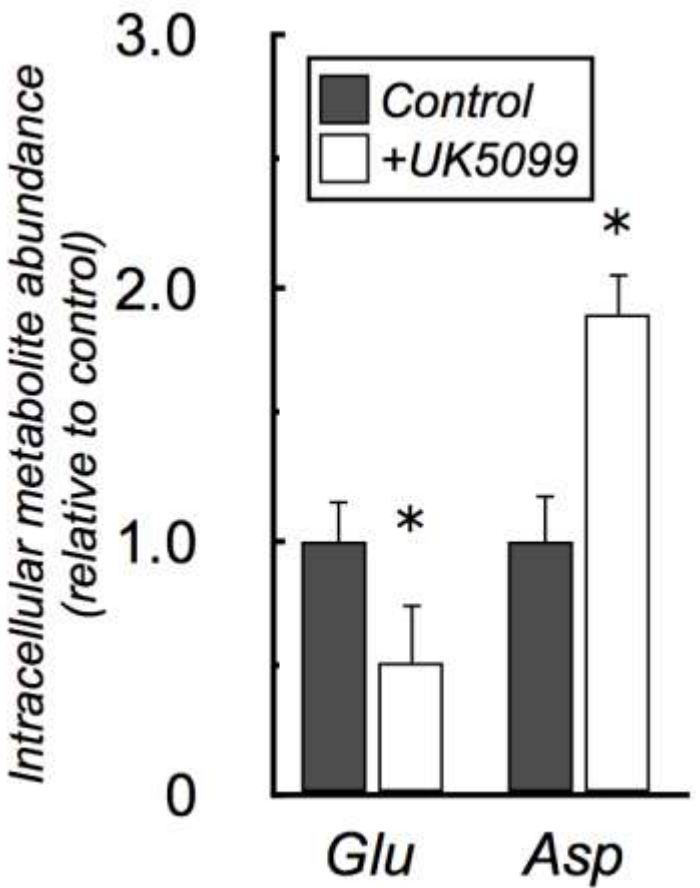




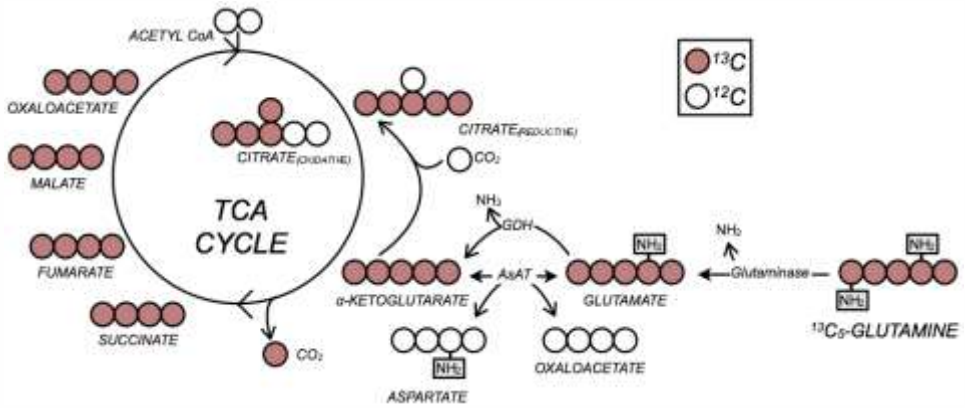
Searching for the protective mechanism of UK5099

Glutamate is generally not considered a metabolic substrate due to its central (and presumably compartmentalized) role in neurotransmission

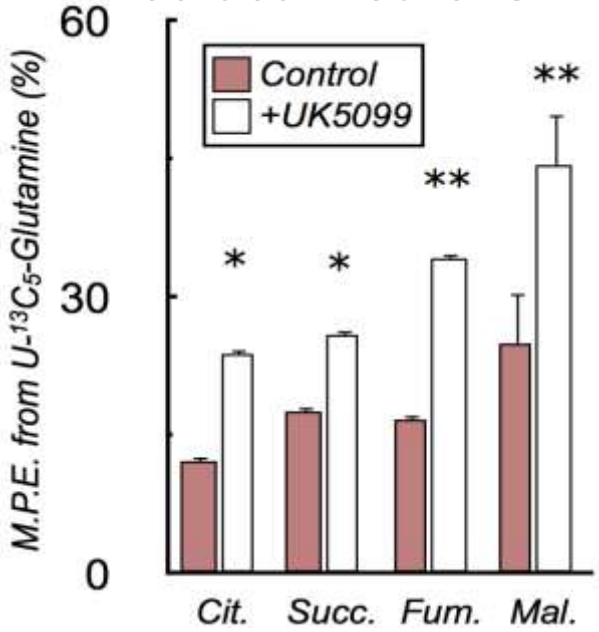




UK5099 selectively increases glutamate oxidation

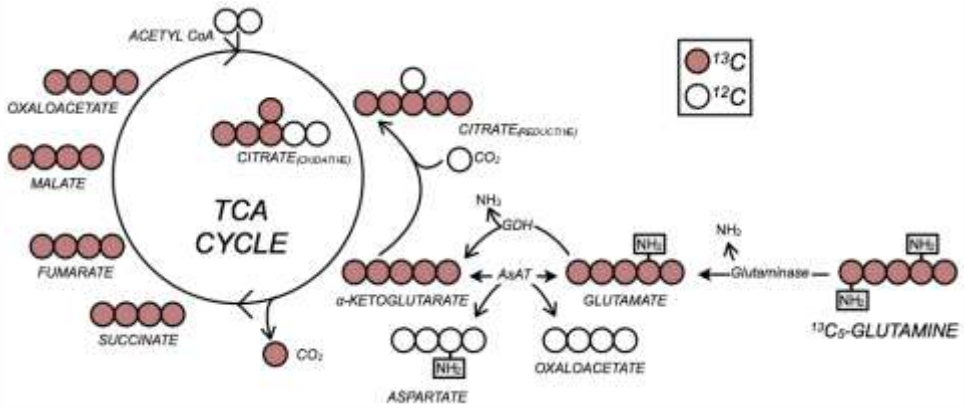


cortical neurons



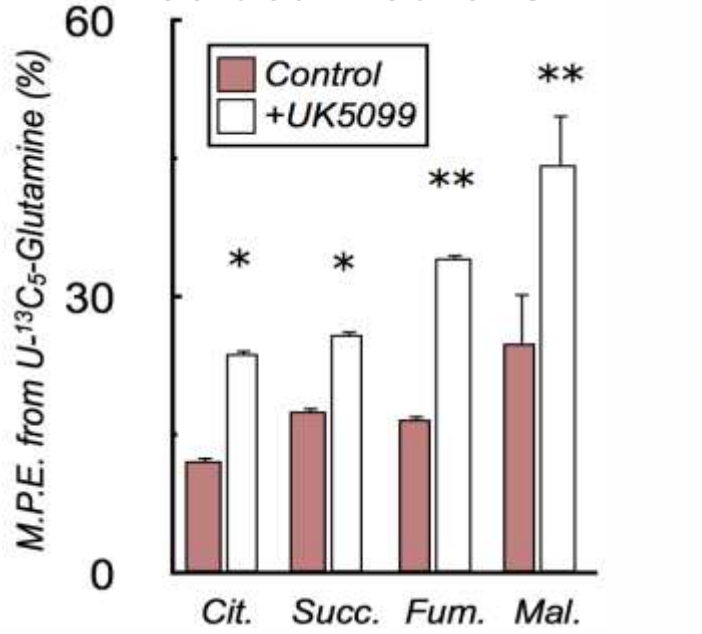
Stable isotope tracing reveals increased glutamine/glutamate oxidation in cells and organotypic slices

UK5099 selectively increases glutamate oxidation

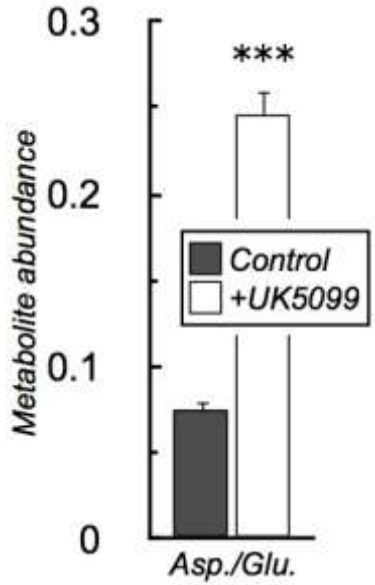


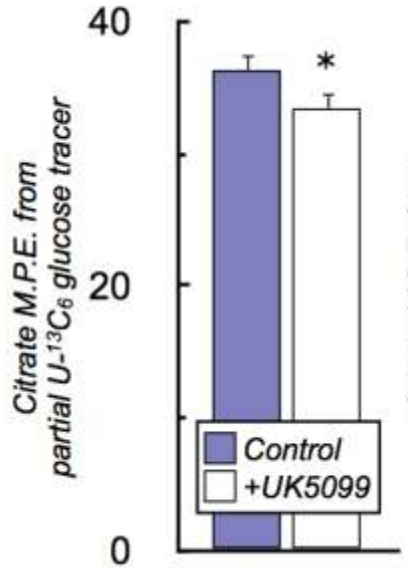
Stable isotope tracing reveals increased glutamine/glutamate oxidation in cells and organotypic slices

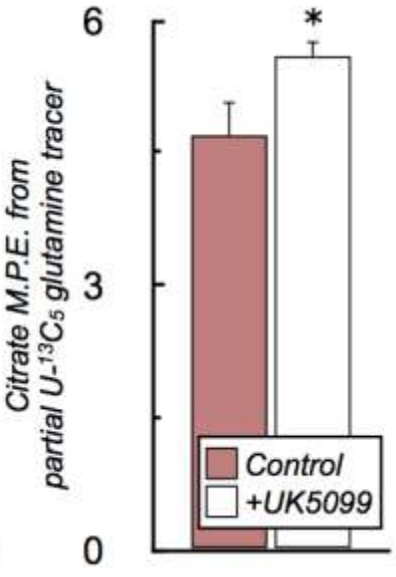
cortical neurons



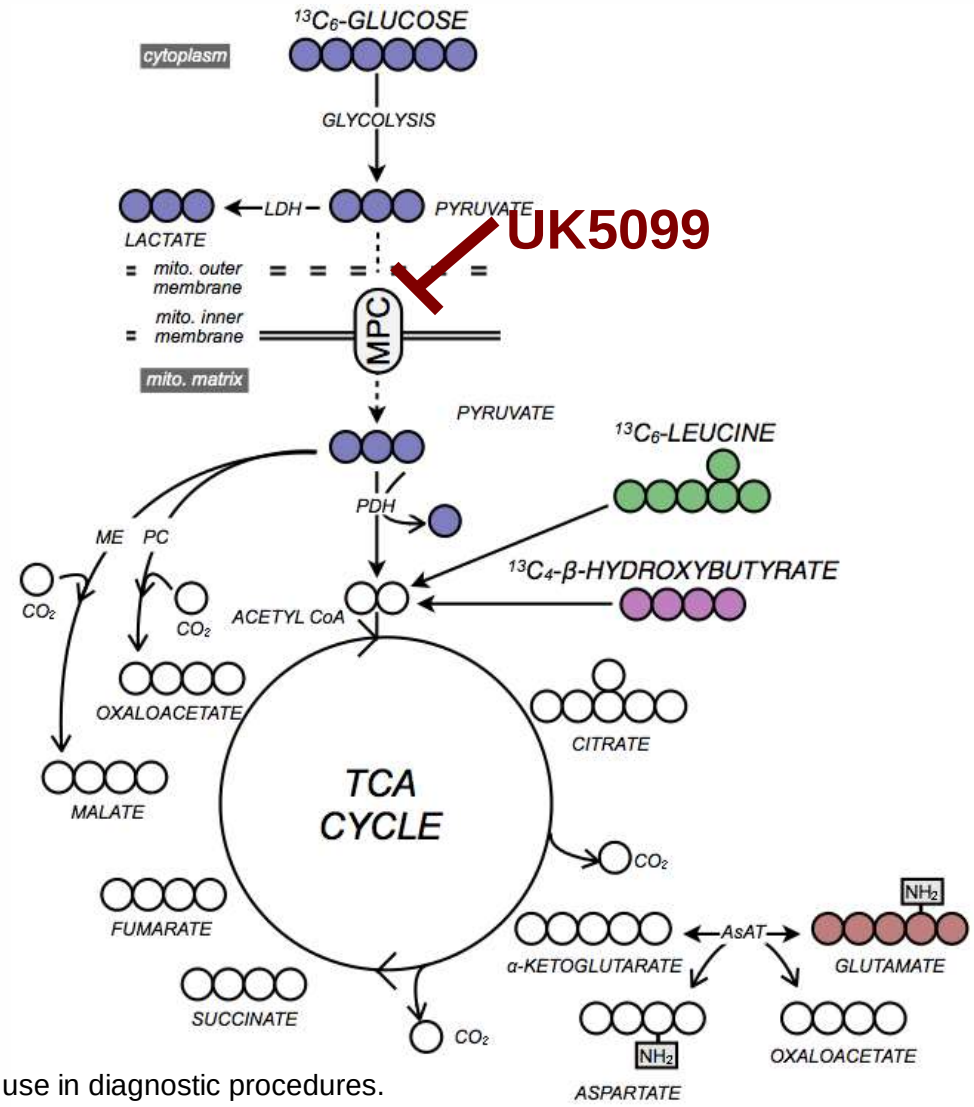
organotypic hippocampal slices





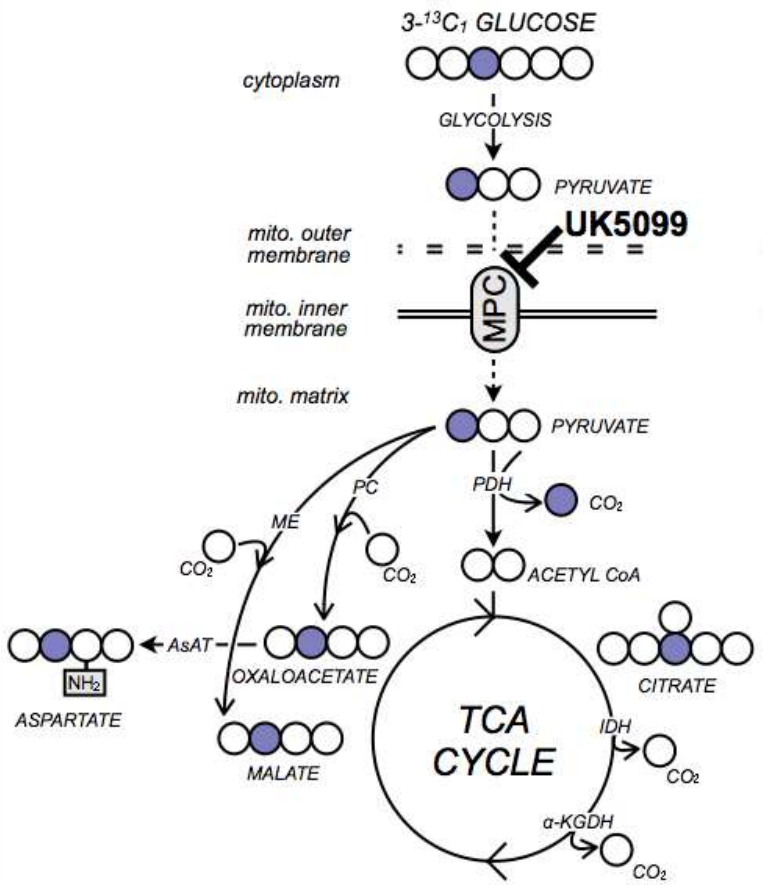


Anaplerosis may explain why glutamate oxidation is selectively increased upon MPC inhibition



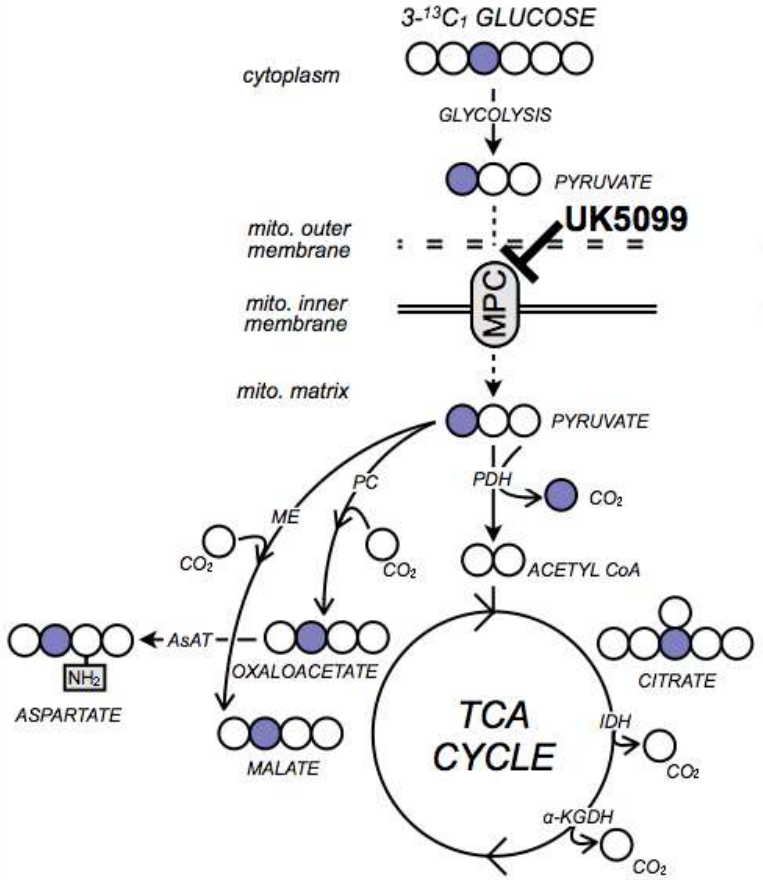
Anaplerosis may explain why glutamate oxidation is selectively increased upon MPC inhibition

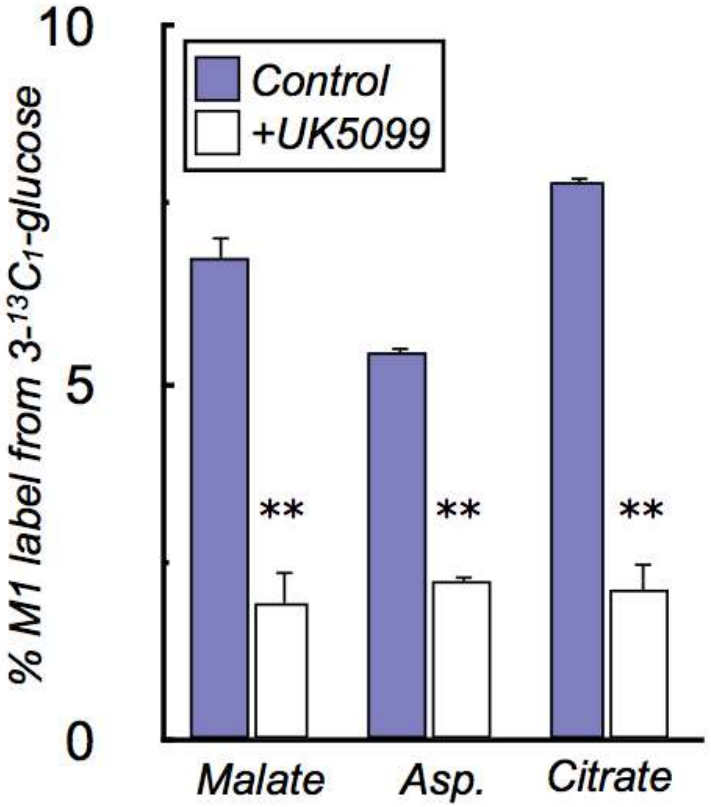
A 3-$^{13}C_1$ glucose tracer can be used to measure CO_2-fixing reactions from glucose that replenish the TCA cycle



Anaplerosis may explain why glutamate oxidation is selectively increased upon MPC inhibition

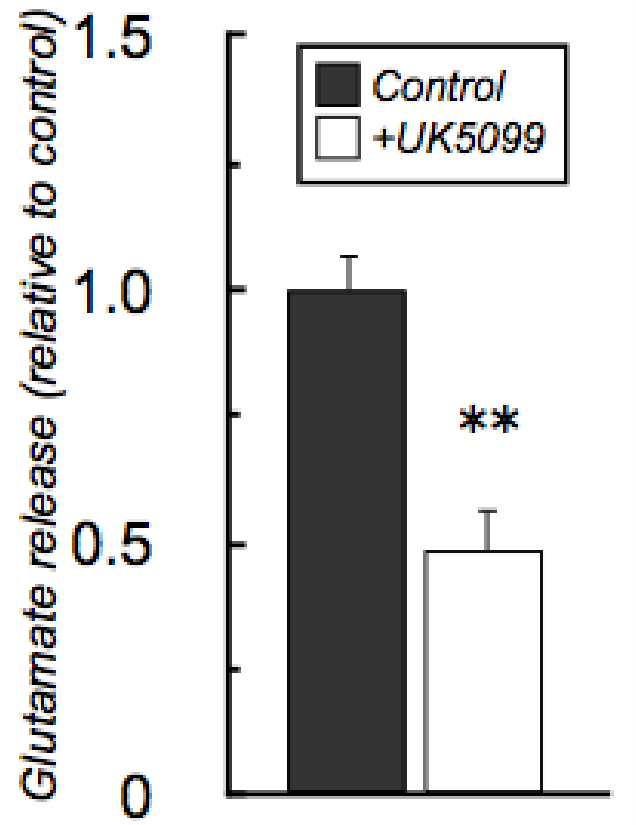
A 3-$^{13}C_1$ glucose tracer can be used to measure CO_2-fixing reactions from glucose that replenish the TCA cycle

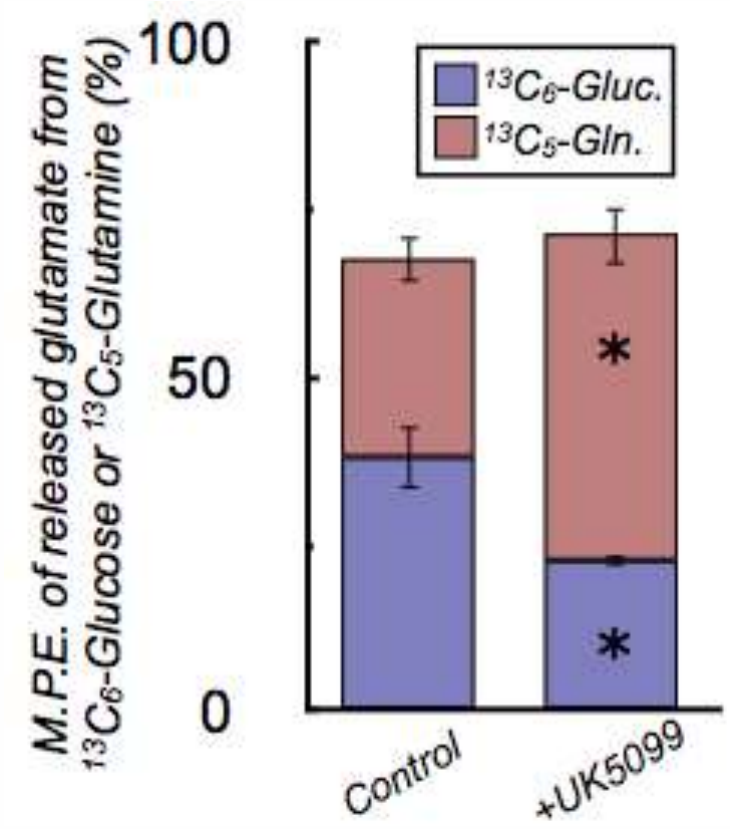




The glutamate mobilized for oxidation is not discrete from the pool apportioned for evoked release

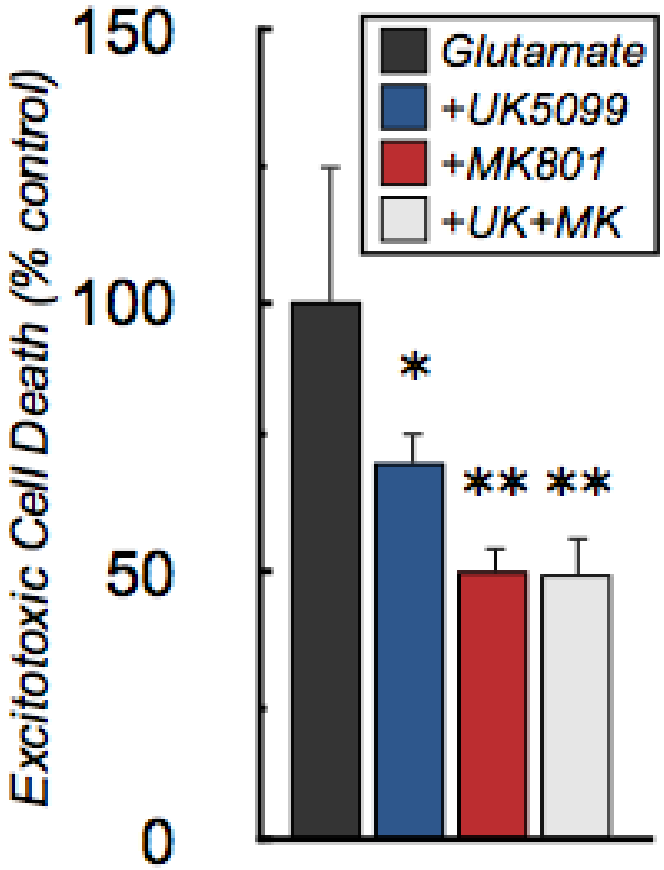
Glutamate released by depolarization from veratridine (prevents Na^+-channel closure) and ouabain (Na^+/K^+-ATPase inhibitor)



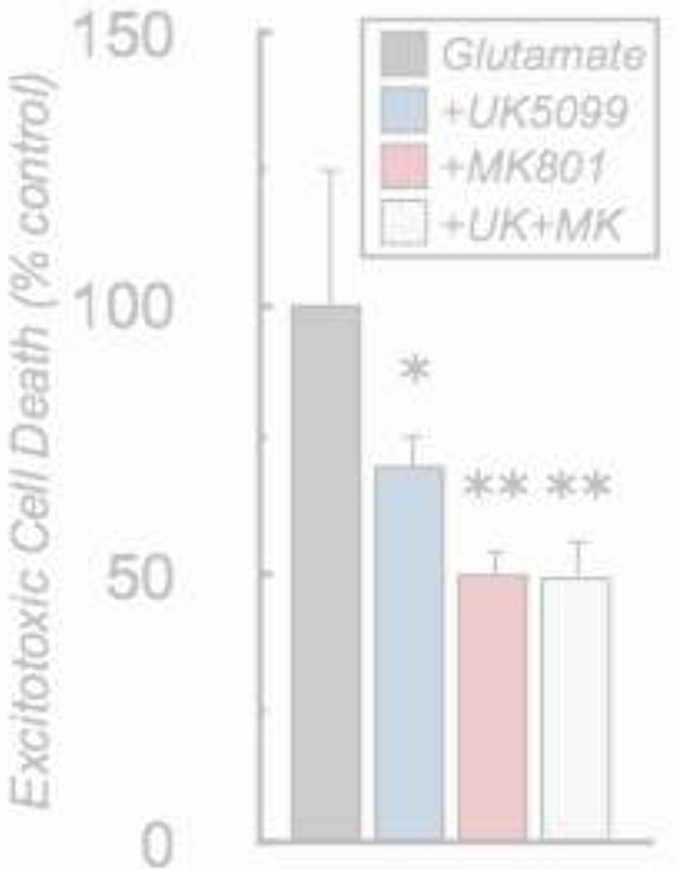


Can attenuated vesicular release explain the protection from excitotoxic injury?

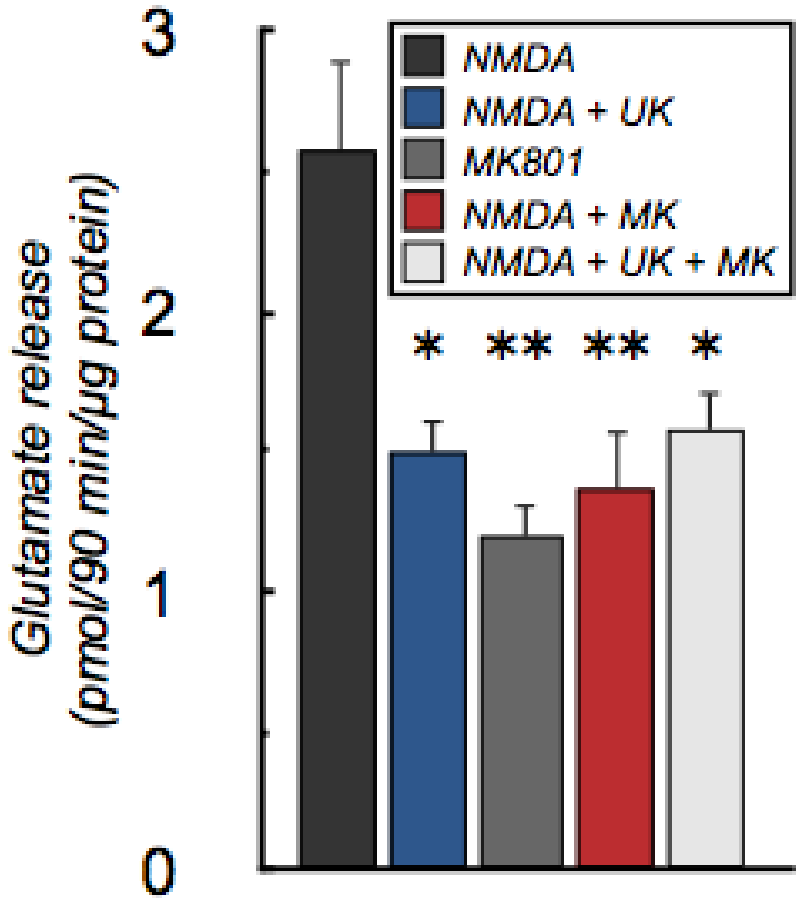
Limiting depolarization-induced glutamate release could minimize the positive-feedback, excitotoxic cascade



Can attenuated vesicular release explain the protection from excitotoxic injury?

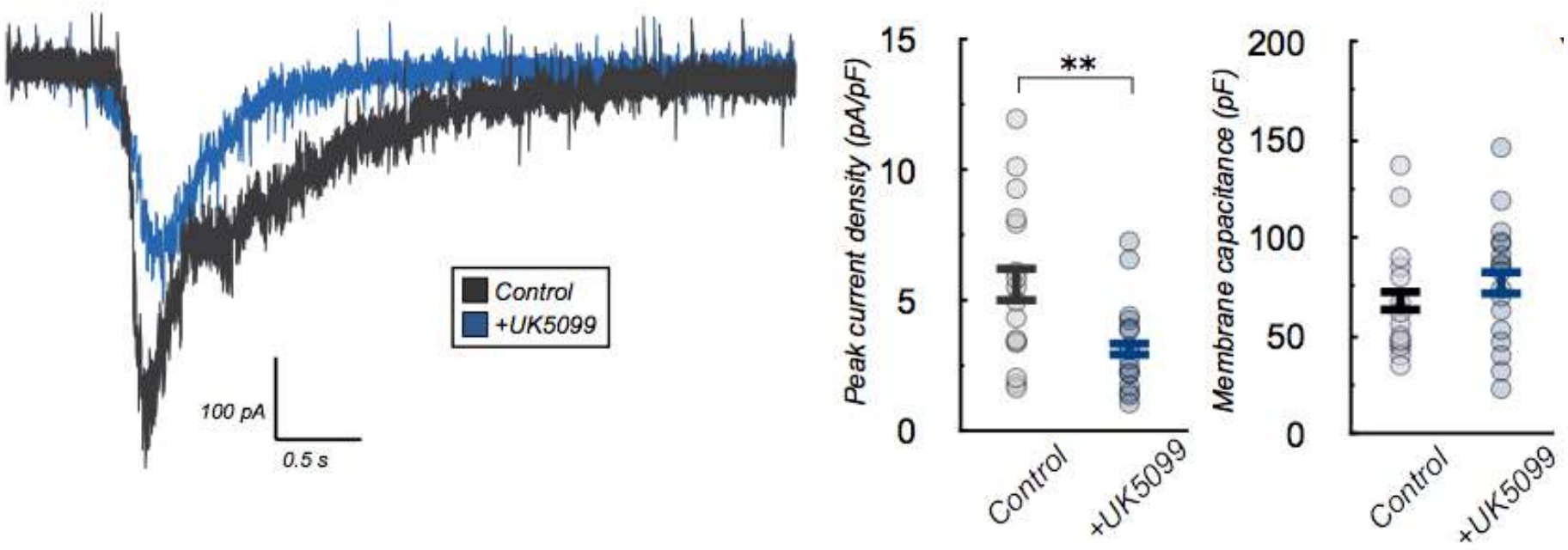


Depolarization induced with a concentration & duration of NMDA that does not compromise viability

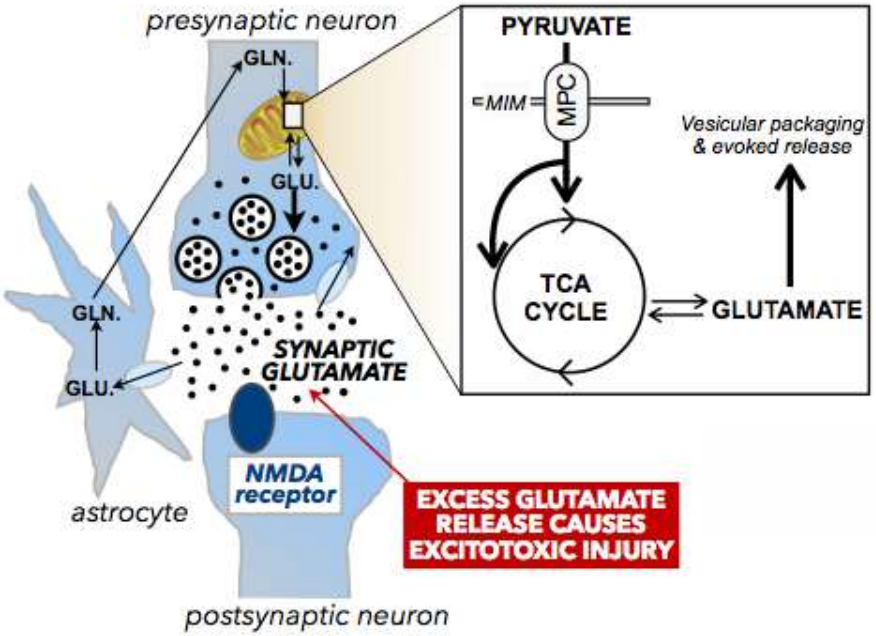


Pyruvate carrier inhibition lowers synaptic glutamate release

UK5099 attenuates excitatory post-synaptic currents in primary mouse pyramidal neurons

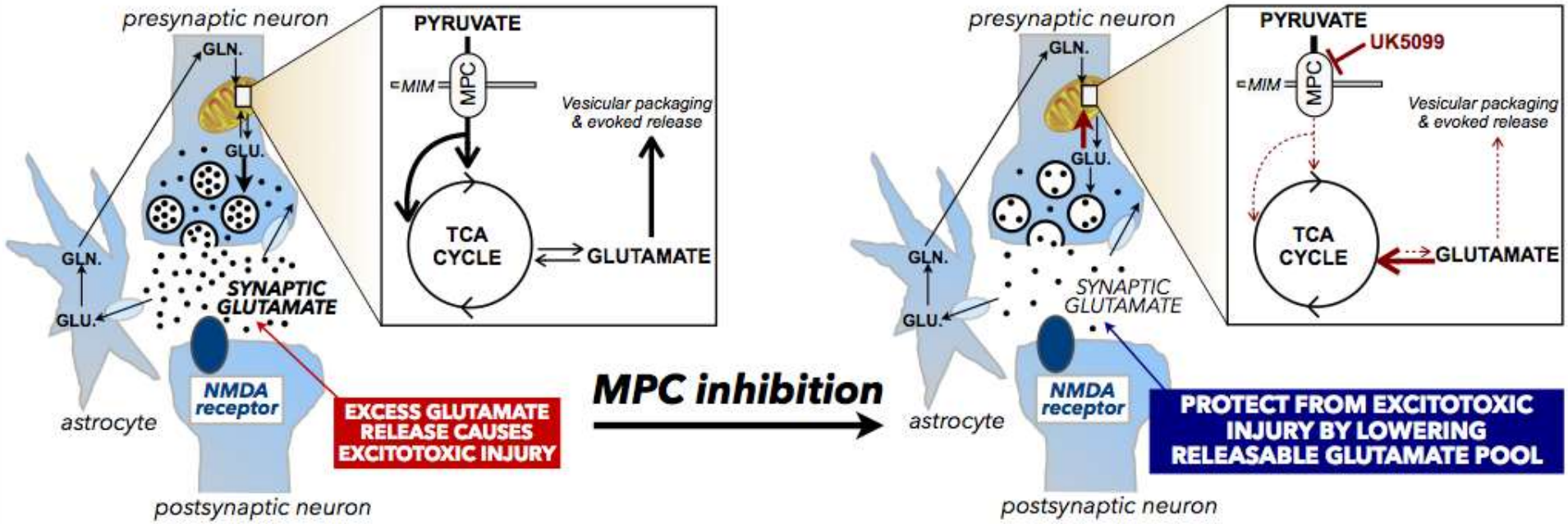


Our model



Divakaruni…and Murphy (2017) J. Cell Biol.

Our model



Divakaruni…and Murphy (2017) J. Cell Biol.

Acknowledgements

UC San Diego

UCSD Jacobs School of Engineering

Anne Murphy

Sandy Wiley Brenda Bloodgood

Alex Andreyev Kelly Martyniuk

Christian Metallo

Martina Wallace

Nate Vacanti

UBC

Lynn Raymond

Caodu Buren

Department of MOLECULAR AND MEDICAL PHARMACOLOGY
University of California, Los Angeles

FUNDING

NIH (NS087611, DK05441, DK81298)

AGILENT TECHNOLOGIES

TEVA PHARMACEUTICALS

Agilent Technologies