The Mitochondrial Target of Thiazolidinediones (mTOT): a New Target for Insulin Sensitizers

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Disclosure: All authors are part owners and/or employees of MSDC.

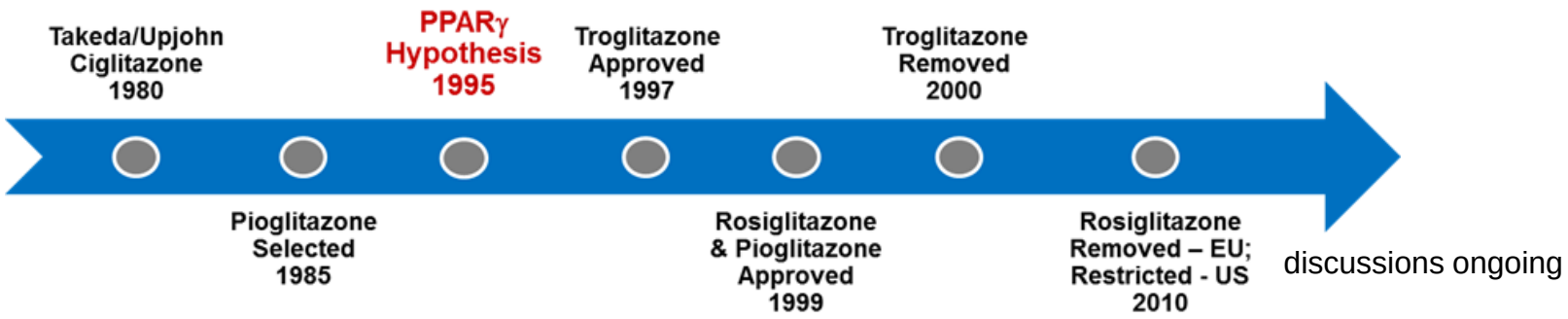
Insulin Sensitizers Have Important Potential

- Insulin sensitizers affect a root cause of type 2 diabetes and would be the preferred choice of treatment if side effects could be avoided.
- Insulin sensitizers have both positive effects on insulin target tissues and on the pancreas, providing the potential to modify the course of the disease and perhaps prevent progression to diabetic complications.
- Recent clinical evidence suggests that early treatment, particularly when combined initially with an incretin therapy might halt the progression of diabetes. [Abdul-Ghani, et al., this meeting 72-OR and 384-OR]

Thus, there is an unmet need for a safe insulin sensitizer that can be used early in the disease.

However, No new insulin sensitizers have been approved since 1999.

The History of Insulin Sensitizers Suggests a New Approach is Needed



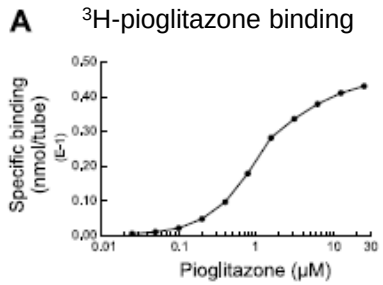
- TZD insulin sensitizers were discovered empirically in the late 1970s.
- Marketed products were selected years before the PPARγ hypothesis. [Colca, J.R. and Kletzien, R.F. (2006). What has prevented the expansion of the insulin sensitizers? Exp. Opin. Invest. Drugs 15:205-210.]
- Pioglitazone is the weakest of activators of PPARγ, but 20 years of clinical experience has proven that it has the best therapeutic profile. [Henry, et al. (2012) PPAR {gamma} Agonists and the Future for Insulin Sensitizers: A report of a debate and presentations on the subject at the 72nd Scientific Sessions of the American Diabetes Association, Philadelphia, 8-12 June, 2012. The British Journal of Diabetes & Vascular Disease, 12: 206 – 210]
- No programs directed at PPARγ agonism have resulted in approved products.
- Compounds with even further reduced activation of PPARγ retain pharmacology. [Colca, et al., Clinical proof of concept with MSDC-0160, a prototype mTOT modulating insulin sensitizer, Clinical Pharmacology and Therapeutics, 93 352-359]
- Effects of insulin sensitizers can be observed in the absence of PPARγ expression. [Chen, Z. (2012) Insulin Resistance and Metabolic Derangements in Obese Mice Are Ameliorated by a Novel Peroxisome Proliferator-activated Receptor -sparing Thiazolidinedione J. Biol. Chem., 287: 23537-23548]

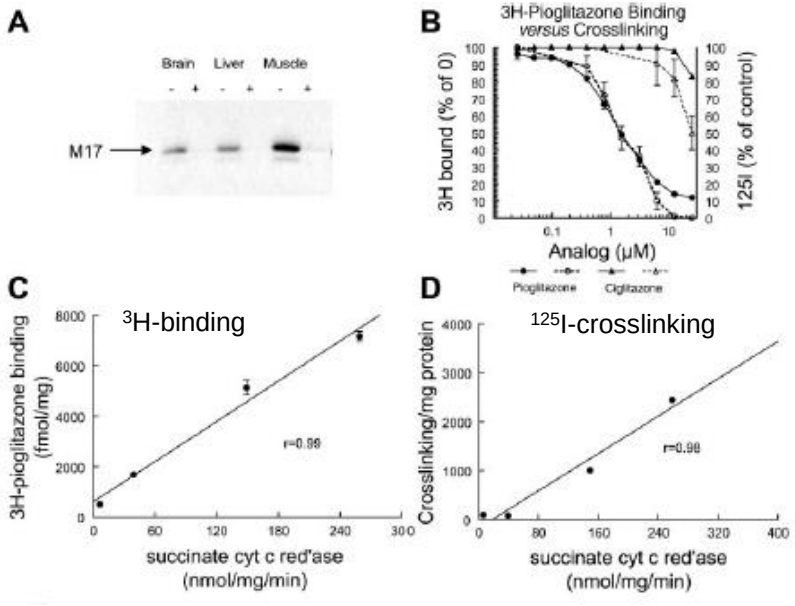
This suggest an undiscovered target for insulin sensitizers

A Specific TZD Binding Site Exists in Mitochondria

Am J Physiol Endocrinol Metab 286: E252–E260, 2004.
First published October 21, 2003; 10.1152/ajpendo.00424.2003.

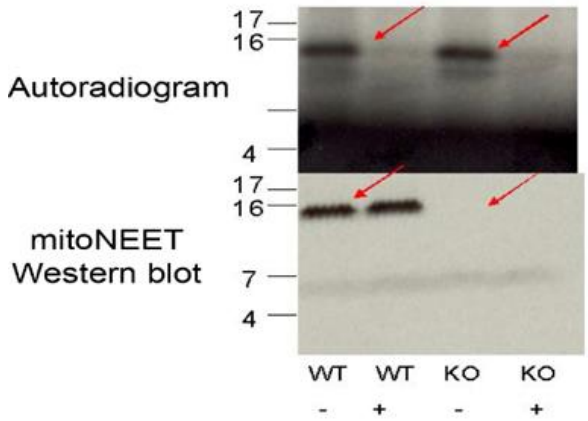
Identification of a novel mitochondrial protein ("mitoNEET") cross-linked specifically by a thiazolidinedione photoprobe

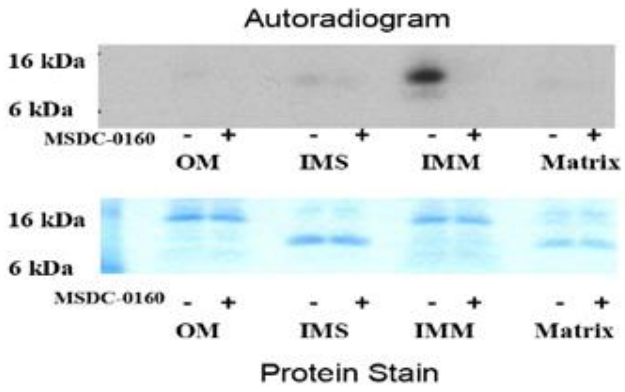




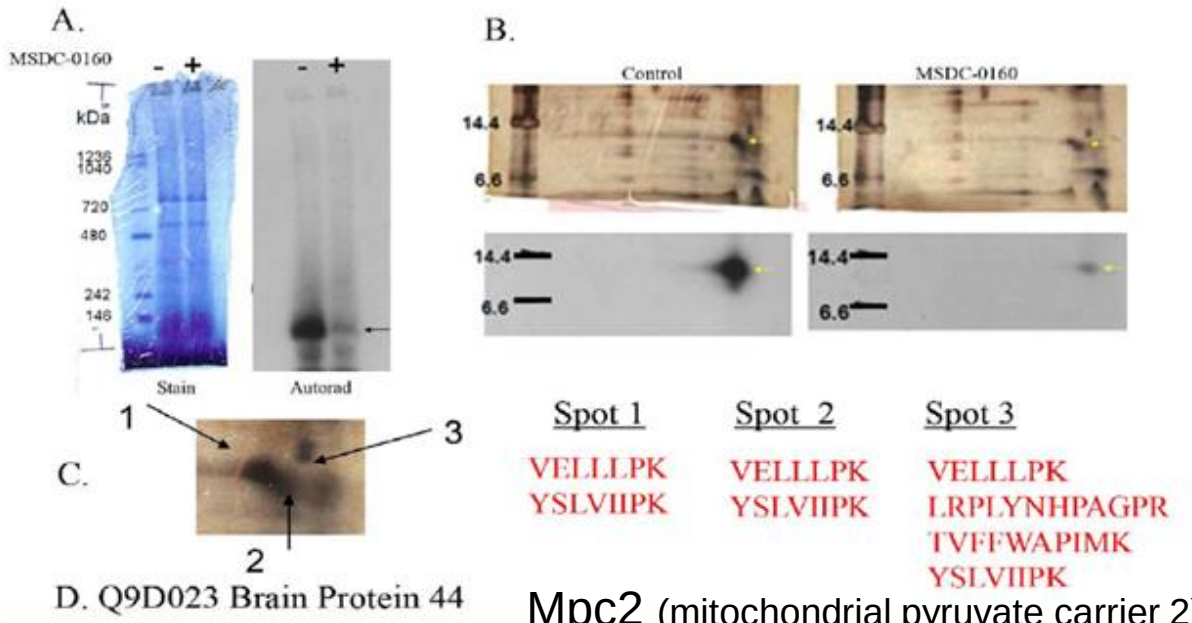
Although the photolabeled protein was once thought to be the OM protein mitoNEET

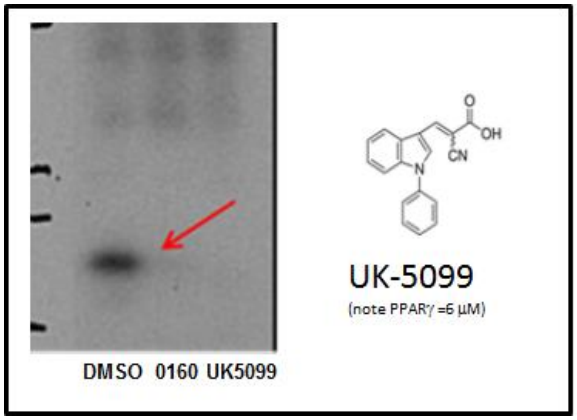
- The crosslinking occurred in mitoNEET null membranes
- Was actually in the inner membrane fraction





The IMM protein that was tagged by the drug analog crosslinker was identified by sequential electrophoresis and mass-spectrometry-based proteomics





Consistent with the ID, the pyruvate carrier inhibitor also blocks crosslinking.

Mpc2 (mitochondrial pyruvate carrier 2)

maaagarglratyhrlmdkvelllpkklrplynhpagprtvffwapimkwglvcagladmarpaeklstaqstvlmatgfiws
ryslviipknwslfavnffvgsagasqlfriwrynqelkskgiq

Science
AAAS

A Mitochondrial Pyruvate Carrier Required for Pyruvate Uptake in Yeast, *Drosophila*, and Humans
Daniel K. Bricker *et al.*
Science **337**, 96 (2012);
DOI: 10.1126/science.1218099

Science
AAAS

Identification and Functional Expression of the Mitochondrial Pyruvate Carrier
Sébastien Herzig *et al.*
Science **337**, 93 (2012);
DOI: 10.1126/science.1218530

PNAS

Thiazolidinediones are acute, specific inhibitors of the mitochondrial pyruvate carrier

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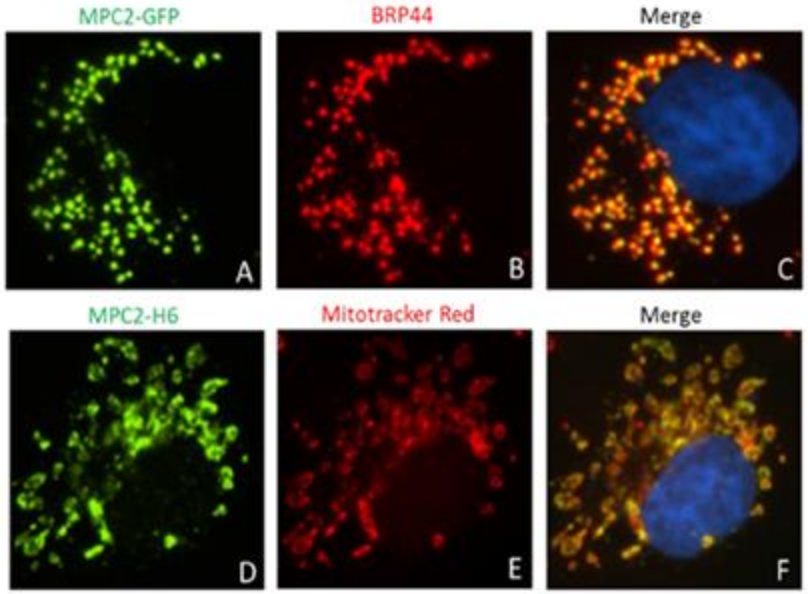
Contributed by Melvin I. Simon, February 21, 2013 (sent for review January 28, 2013)

ment company

Mpc2 was expressed with a C-terminal GFP or His6

- The protein was expressed in the mitochondria (overlay Mitotracker red)



Membranes from WT or His6 construct cells were subjected to photaffinity crosslinking and both WT and extended protein were crosslinked in a similar fashion

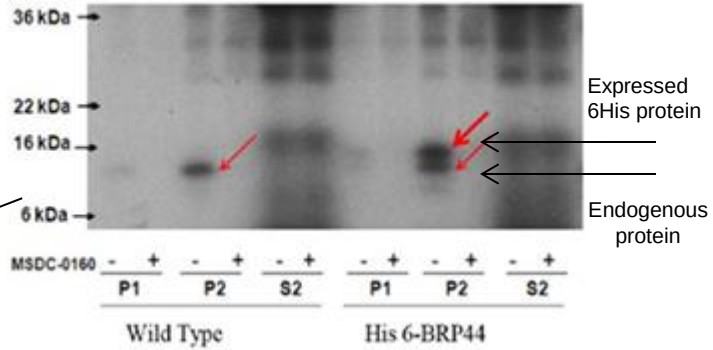
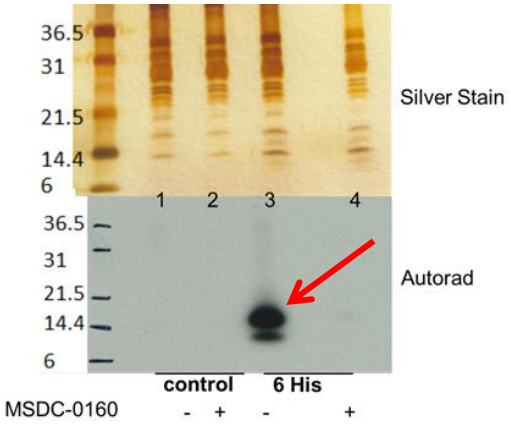


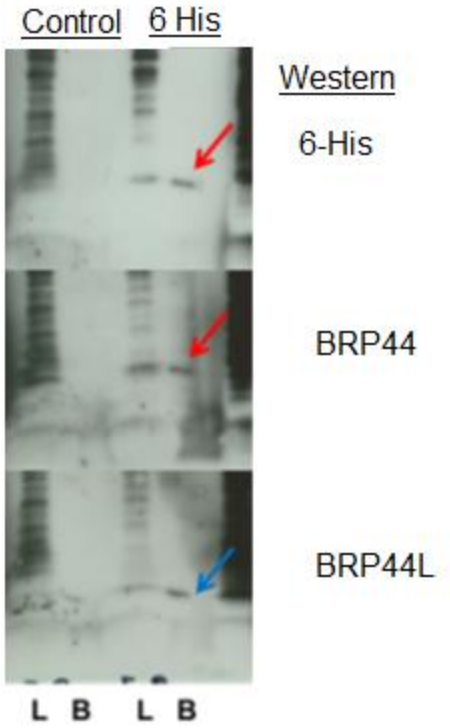
Photo affinity crosslinked membranes were immunoprecipitated (IP) with anti-6His



- Autoradiogram of gels shows that the crosslinked protein is selectively from IP'ed from His6 sample.
- These samples were also submitted to Western blots and proteomics.

Other Associated Proteins

Western Blots of IP of Mpc2-6His



L= Loaded
B= Bound

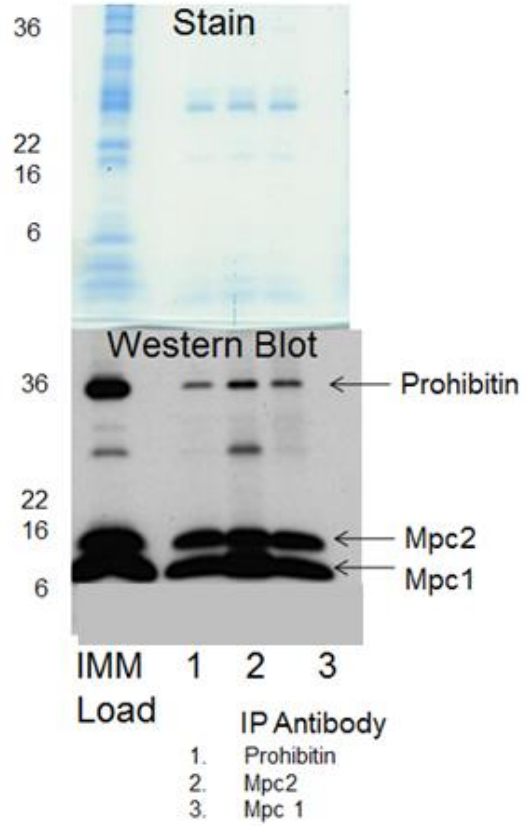
Possible involvement of Prohibitin

Proteomics of gel slices from HEK293 cells also identified Prohibitin (P35232)

Prohibitin was also identified from blue native gel samples from both brown adipose cells (BAT) and Liver.

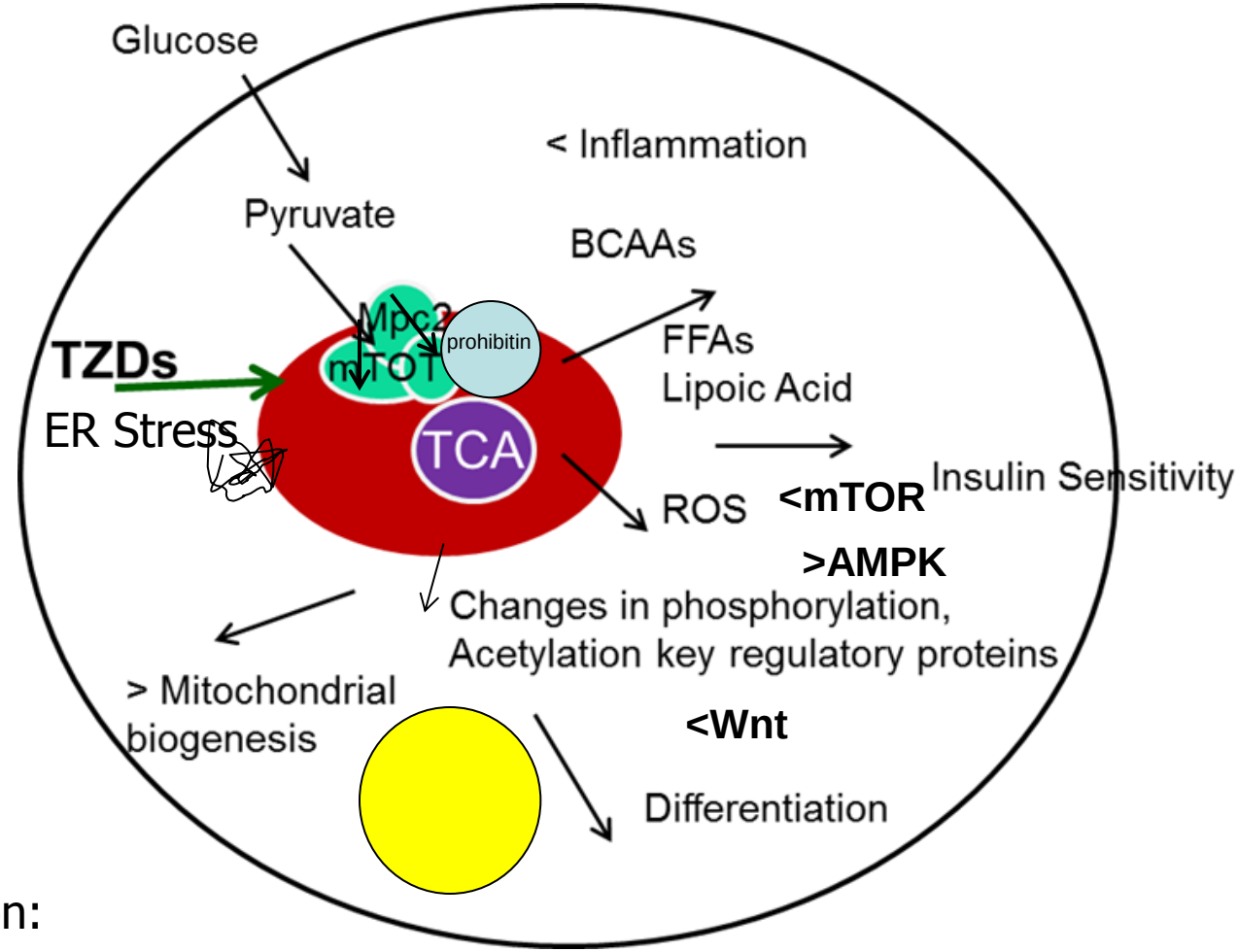
Immunoprecipitation of solubilized (0.25% n-Dodecyl β-D-maltoside) liver IMM fractions by antibodies to either Mpc2, Mpc1, or prohibitin pulled down portions of all three proteins.

Western Blots of IP of Liver IMM



- Vessal et al (2006) Prohibitin attenuates insulin-stimulated glucose and fatty acid oxidation in adipose tissue by inhibition of pyruvate carboxylase. FEBS J 273(3): 568-76.
- Ande et al (2012) Prohibitin has an important role in adipocyte differentiation. Int J Obes (Lond), September 1, 2012; 36(9): 1236-44.
- Liu et al (2012) Mitochondrial dysfunction and adipogenic reduction by prohibitin silencing in 3T3-L1 cells. PLoS One 7(3): e34315

mTOT Hypothesis – Coordination of mitochondrial metabolism can logically orchestrate the insulin sensitizing pharmacology observed.

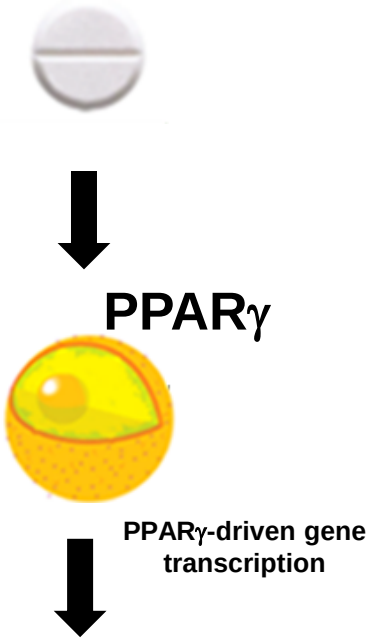


Coordination:

- Nutrient sensing pathways (<mTOR; >AMPK)
- Redox signals (enzymatic activity, transcription factors, etc)
- Other mitochondrial functions

Implication for New Insulin Sensitizers

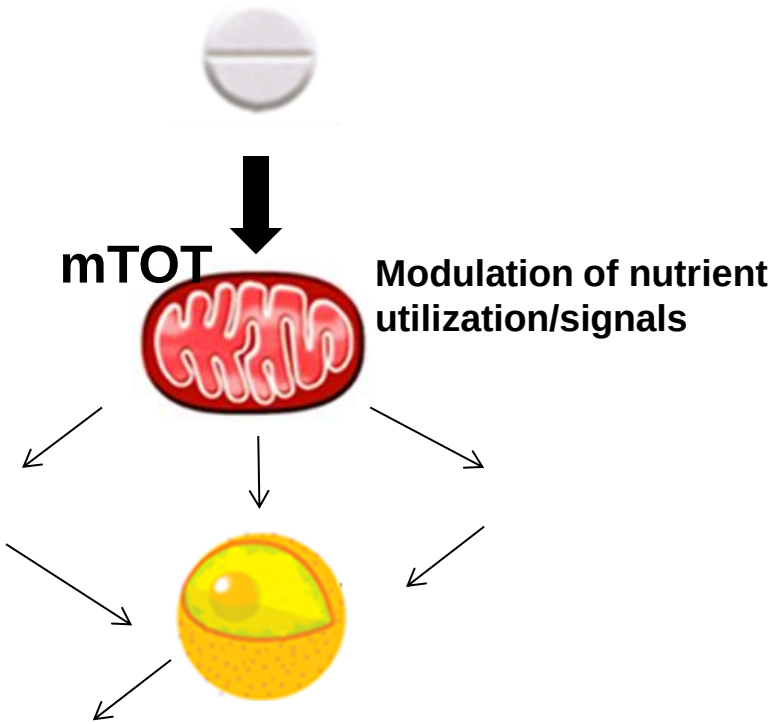
Hypothesis followed since 1995



Increased gene expression favoring

- **Lipid synthesis and storage** – leading to improved insulin action
- Increase in white adipose tissue
- Volume expansion and other PPARγ-driven side effects

New Hypothesis



Coordinated metabolic response (cell specific)

- **Increased fat oxidation**
- Differentiated BAT; Browning of WAT
- <mTOT; >AMPK ….insulin sensitivity and anti-inflammation
- β-cell phenotype (2195-P)