Mechanism of aging and Dementia: similar or different

תהליכי הזדקנות ודמנציה: האם הם דומים תמיד?

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The way of life of the ancient man was concentrated in obtaining food and escaping from predatory animals and bad weather

Researchers estimate the life expectancy of man in the Stone Age at 18 years and of man in the Bronze Age at 33 years. The life expectancy of Neanderthal man, which became extinct 40,000 years ago, is estimated at about 20 years.

Regardless of our modern way of life, biologically there is no significant difference between ancient man and us. Our metabolic system still requires minimal amount of food achieved through physical work.

These facts did not change because refrigerators were invented, together with antibiotics, vaccines, and modern houses.

However, the new life stile resulted in increased life span. It was then that we started to worry about aging and its consequences, such as loss of memory and cognitive decline

Aging and Neurodegeneration: Current dogma

Aging is the greatest risk factor for the development of neurodegenerative disease

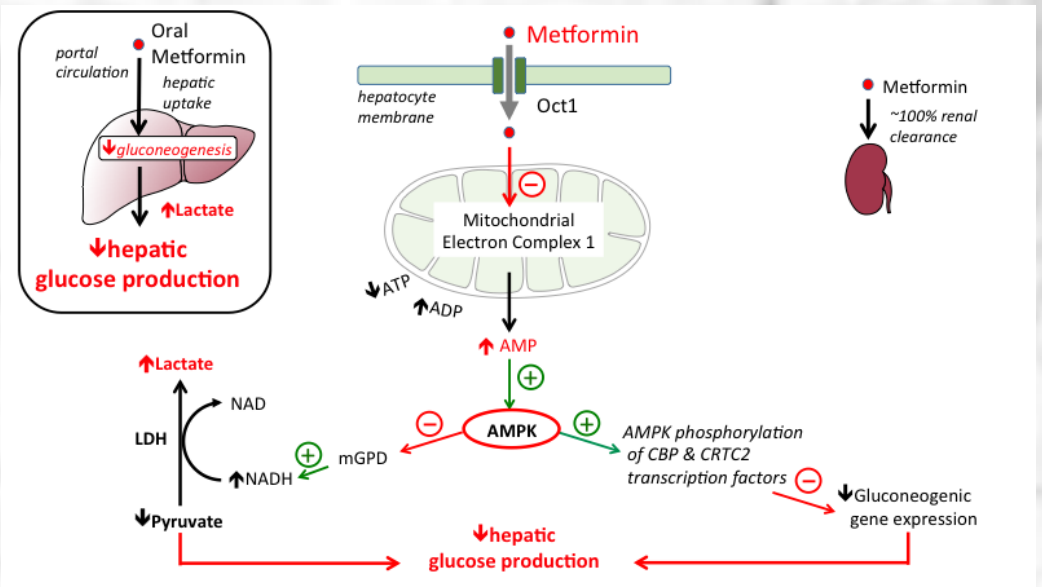
In the inherited forms of these diseases, while the causing mutations are present since birth, symptoms appear decades later, indicating a role for aging in disease appearance. Based on this observation, it may well be that changes taking place during "normal aging" make brain cells susceptible to the disease-causing mutations.

If so, then delaying aging may/should postpone the onset of neurodegenerative diseases.

Therefore, compounds that have been shown to extend longevity such as Metformin, rapamycin, resveratrol, N-acetyl-L-cysteine, curcumin, could delay the onset of disease. On the other side, if you live longer, there are more opportunities to develop age related diseases.

In this project, we compared the effects of Metformin (anti diabetic and anti-aging drug) to those of Granagard (a neuroprotective anti-oxidant) proven to delay the progression of disease in genetic CJD and AD mice

METFORMIN



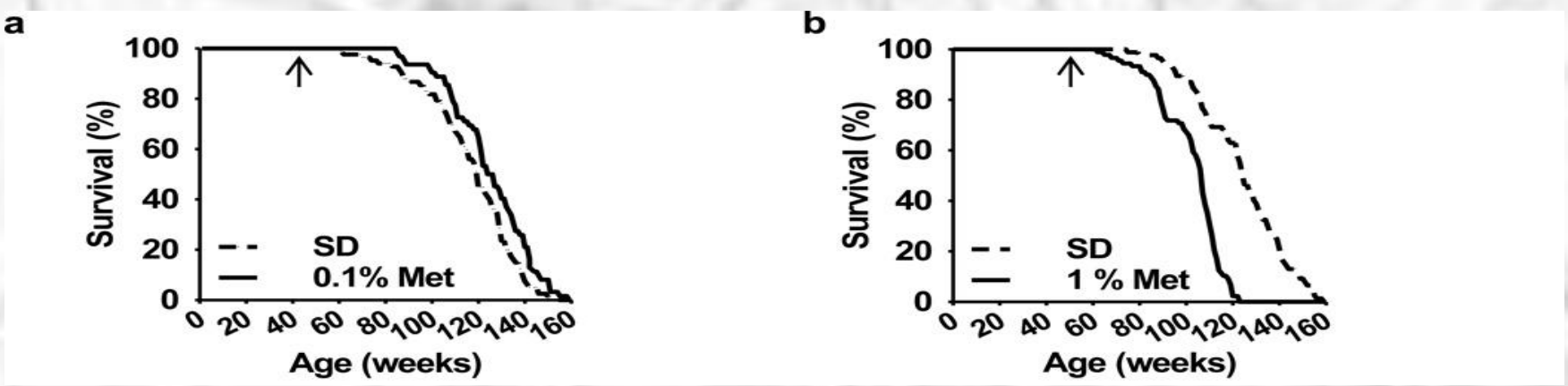
Anti Diabetic

Metformin's most important effect in treating diabetes is the inhibition of gluconeogenesis, the hepatic pathway for the production of glucose

Nat Commun. 2014 Jan 31.

Metformin improves healthspan and lifespan in mice

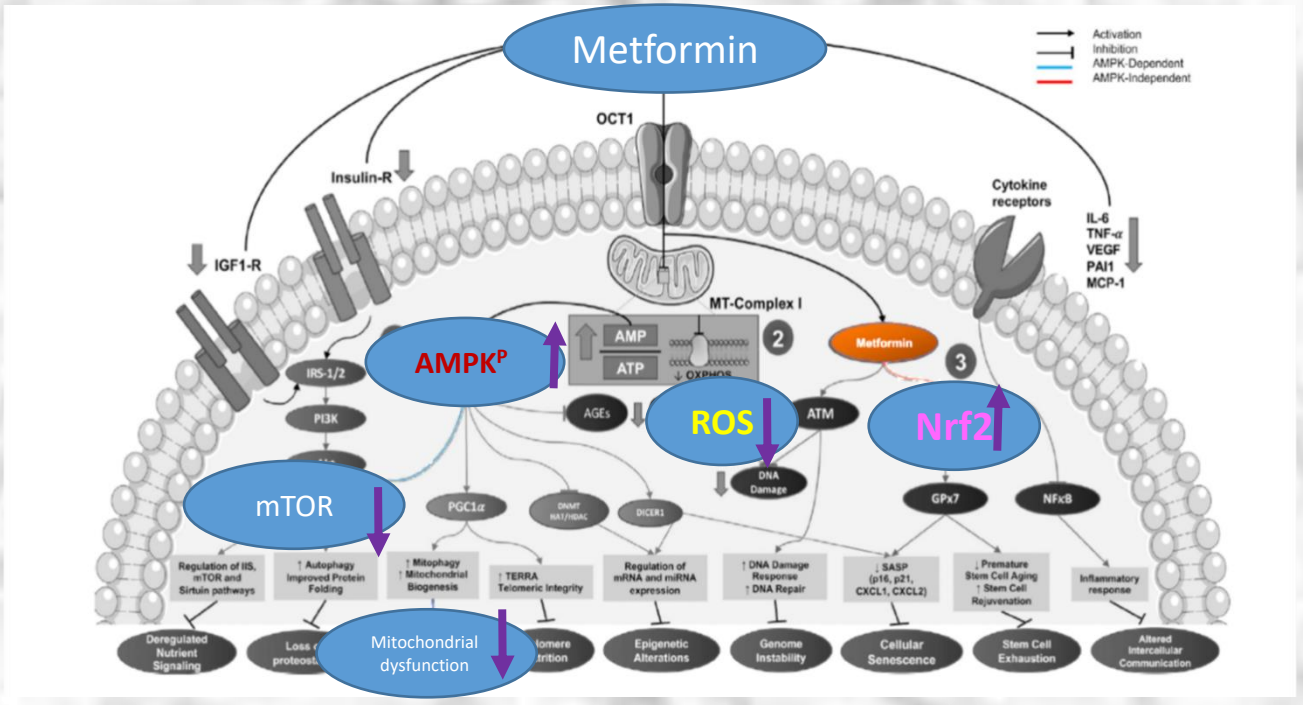
ANTI-AGING



Anti-aging pathways maintain the energy status and the antioxidant position of cells. Metformin activates AMPK dependent or independent (Nrf2) pathways

AMPK is a master energy sensor whose activation helps maintain cellular energy levels. AMPK acts by phosphorylating key enzymes in metabolic pathways as well as transcriptional factors and cofactors. This protein **plays an important role** in cell survival and organismal longevity through modulation of **energy homeostasis and autophagy**.

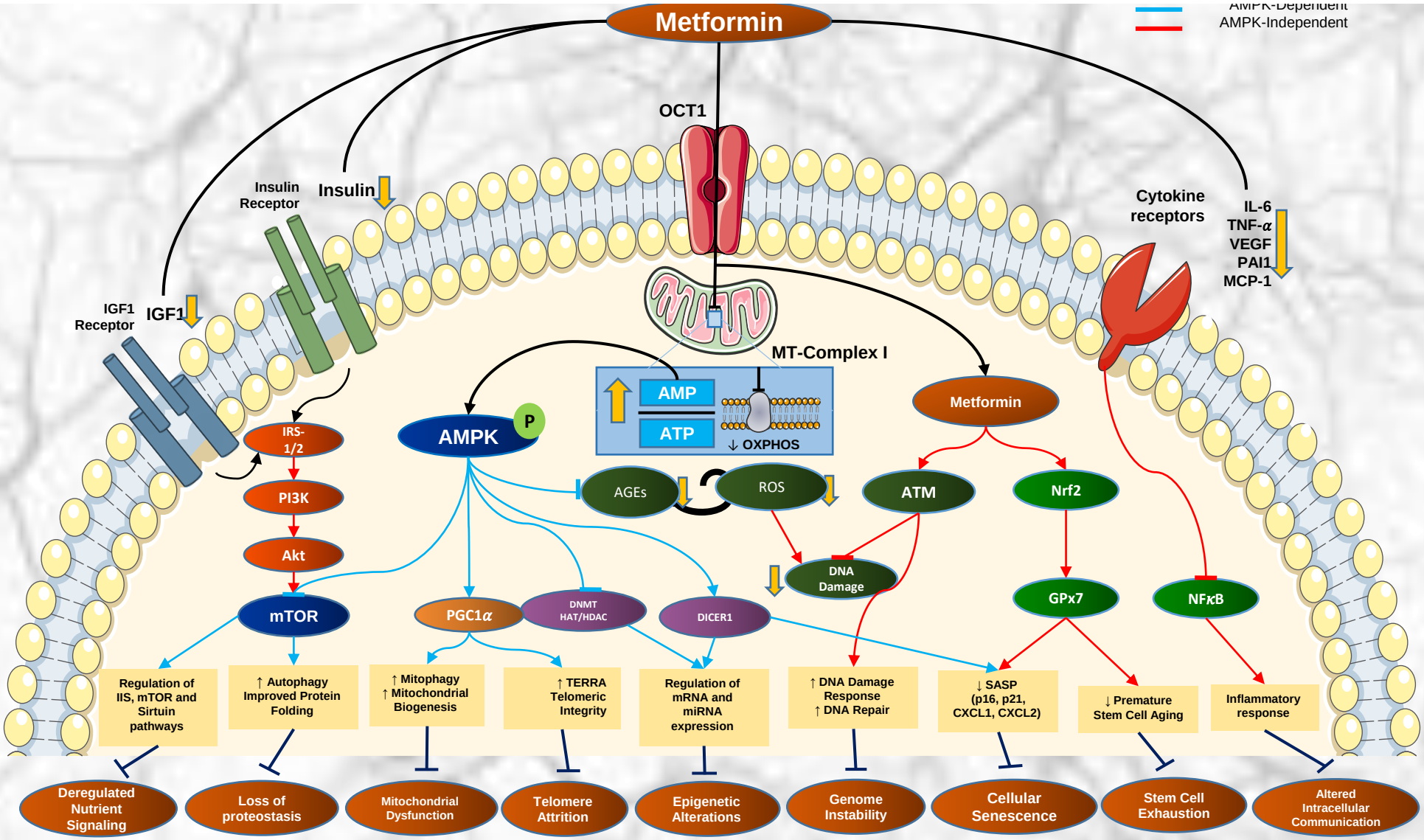
While in some experiments **Metformin** delays deterioration of AD mice, it was also shown that high levels of AMPK may increase the generation of both **intracellular and extracellular A-beta species.**



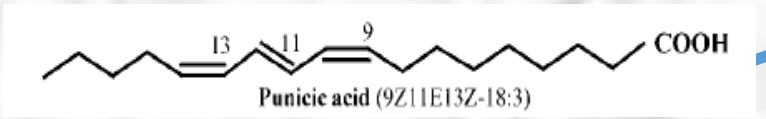
A Large human study: **TAME** (**Targeting Aging with Metformin**) trial, was set to asses the anti aging properties of Metformin, is in progress.

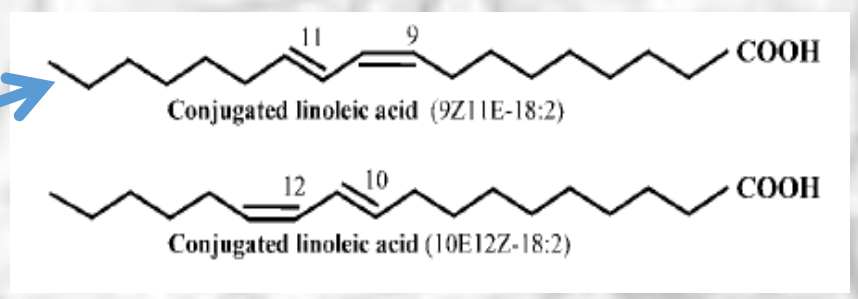
Kulkarni et al., Benefits of Metformin in Attenuating the **Hallmarks of Aging**, Cell Metabolism (2020),

Lopez-Otin, (2013). The hallmarks of aging. Cell *153*, 1194-1217



GranaGard: a nanodroplet formulation of Pomegranate Seed Oil





PSO contains high concentrations (80-90%) of Punicic acid (PA), also known as Omega 5), a polyunsaturated fatty acid with three conjugated double bonds, considered one of the strongest natural antioxidants

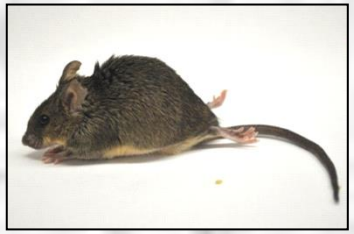
In-vivo PA was shown to metabolize into 9c, 11t conjugated linoleic acid (CLA), both an antioxidant and a μ-calpain inhibitor, shown to inhibit Aβ oligomerization in Alzheimer's disease cellular models

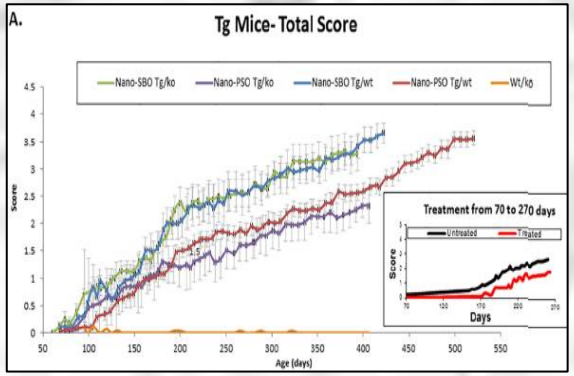
Testing the effect of GranaGard in animal models of disease

Activation of anti AGING hallmarks?

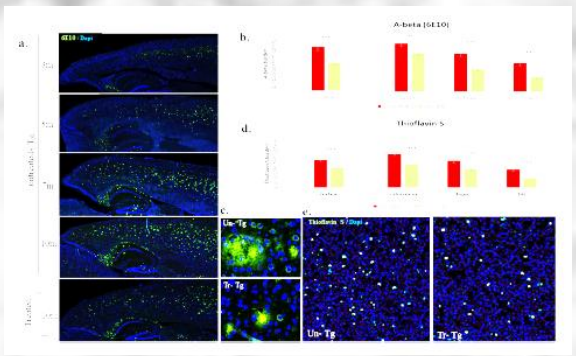
Increased survival of transplanted and endogenous stem cells In CJD mice/ In EAE mice?

CJD E200K

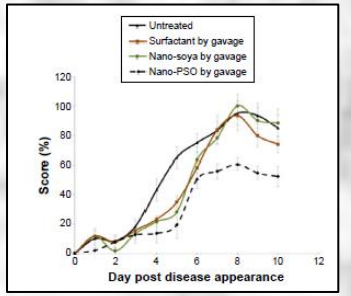




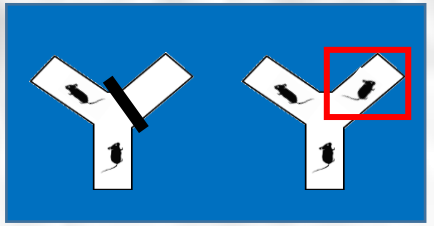
Alzheimer's 5XFAD

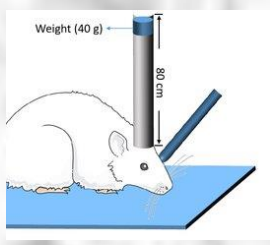


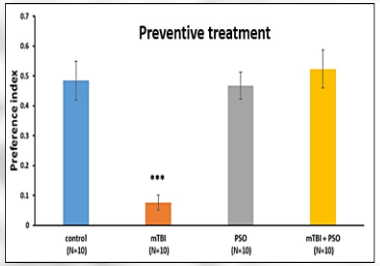
EAE: MS

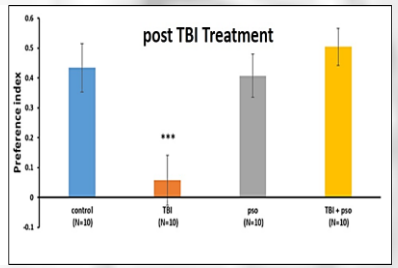


Brain Injury









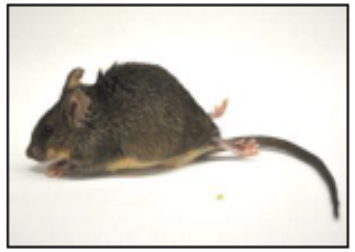
Nanomedicine 2014 1353-63
Int J Nanomedicine 2015 7151-74
Sci Rep 2019 Dec 5;9(1):18437
Neurobiology of Disease 2017 140-147
Neurobiology of Disease 2018124:57-66
Neurobiology of aging 2020

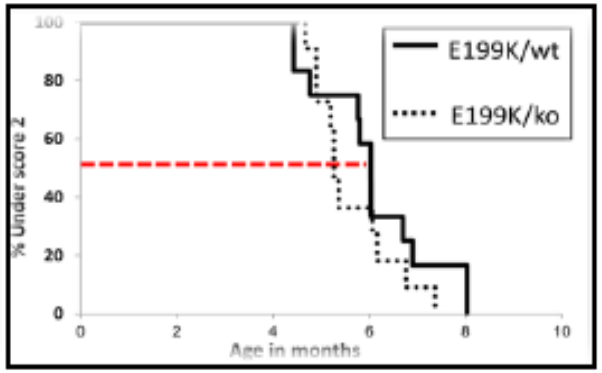
TgMHu2ME199K mice model for E200K CJD patients (Libyan Jews)

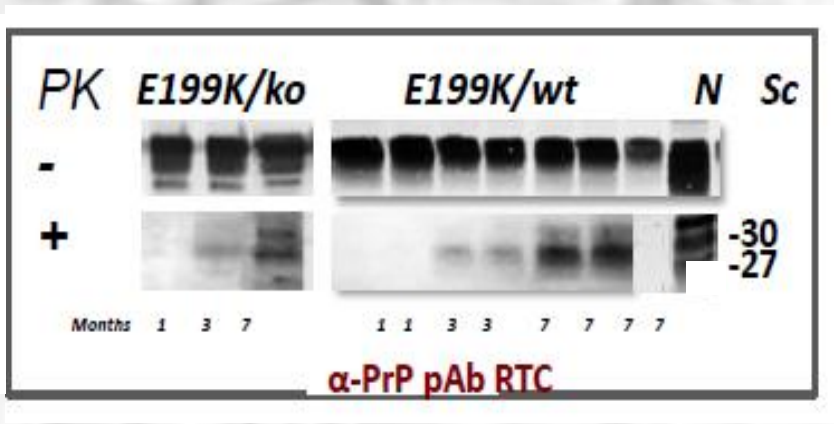
Generated in FVB mice comprising PrP chimeric human/mice at UCSF by Prof Zeev Meiner

"Revived" from frozen sperm into C57B mice in our laboratory

mouse -human-mouse *PRNP* chimera expressing an E199K mutation, corresponding to the E200K mutation in humans, linked to the most prevalent genetic prion disease







Fried et al Neurobiol of aging 2018

Keller et al Neurobiol of diseases 2019

Canello et al, Neurobiology of disease 2012

Friedman Levy et al, Plos One 2013

Friedman Levy et al, Plos Pathogens, 2011

Fried at al neurobiology of aging 2020

Binyamin et al Neurobiol disease 2018

Cohen et al, Plos One 2013

Binyamin et al Neurobiol Aging. 2022

Friedman Levy et al, Human Mol Genet, 2014

Fainstein el al, Front Neurosci, 2016

Mizrahi el al, NanoMedicine 2014

Comparing the effects of Metformin and Granagard on aging and neurodegenerative markers in TgMHu2ME199K mice modeling for genetic CJD

Groups of C57BL and TgMHu2ME199K mice were treated for either 2 weeks or 4 months either with Metformin or Granagard. Treated and untreated TgMHu2ME199K mice were followed for their neurologic score. At the end of the treatments, lungs, muscles and brains were collected from all groups for biochemical and pathologic evaluation of aging and prion disease hallmarks

Neurodegeneration

Will Granagard work here?

Will Metformin work here?

General Aging hallmarks

Specific disease hallmarks

- Telomere Attrition
- Epigenetic Alterations
- Loss of proteostasis
- Deregulated Nutrient Signaling
- Mitochondrial Dysfunction
- Cellular Senescence
- Stem Cell Exhaustion
- Altered Intracellular Communication

↓ pAMPK

↑ IGF-1

↓ cox4-1

↓ Hif-1

↓ NRF-2

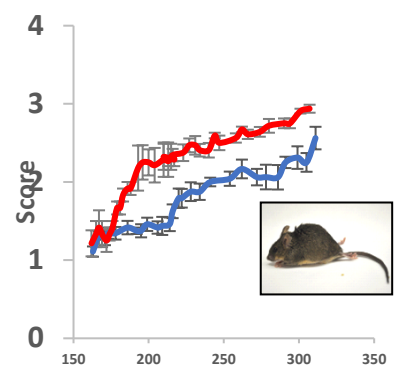
↓ Stem-Cells production

↓ Inflammatory response

Disease advance

Key Protein Accumulation PrP ?

GAGs accumulation Brains, urine?



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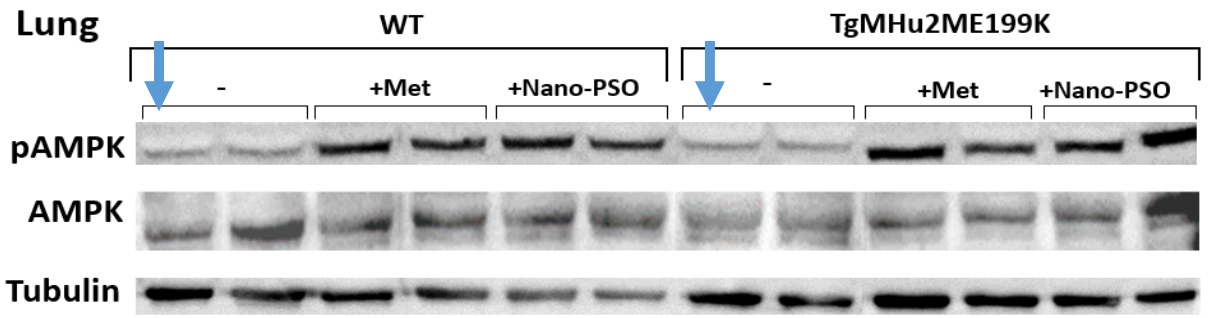
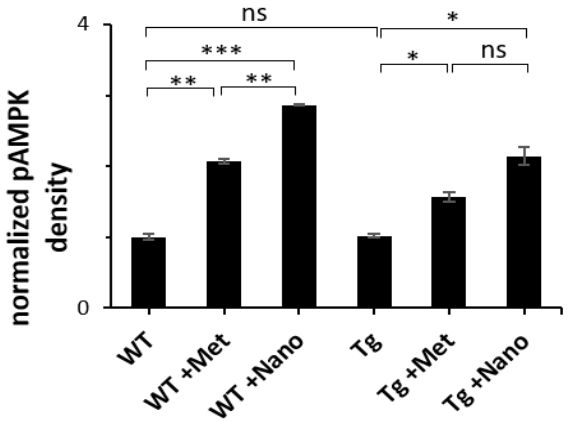
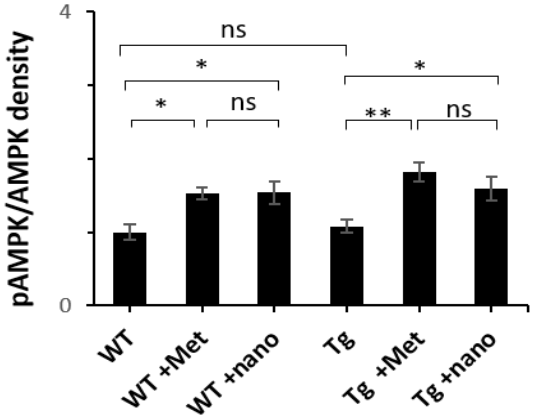
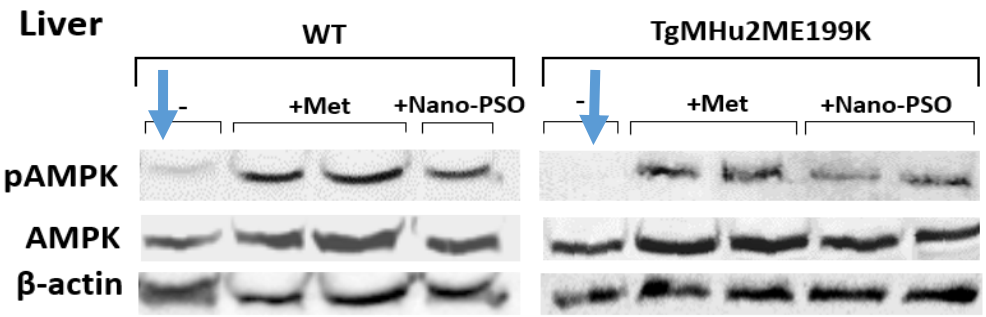
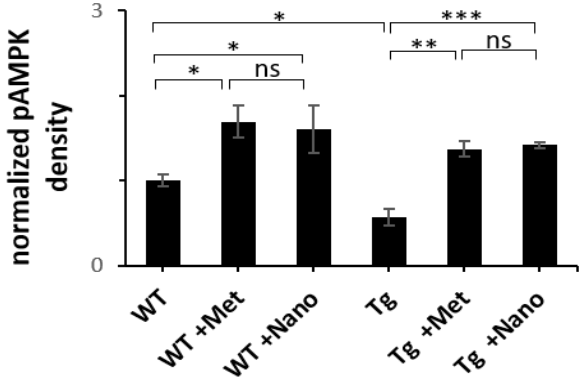
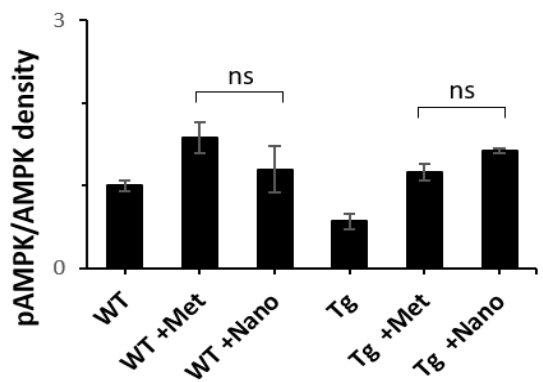


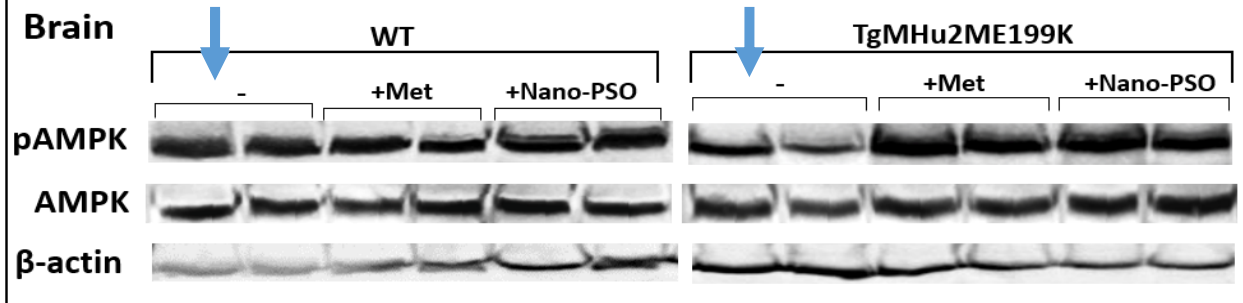


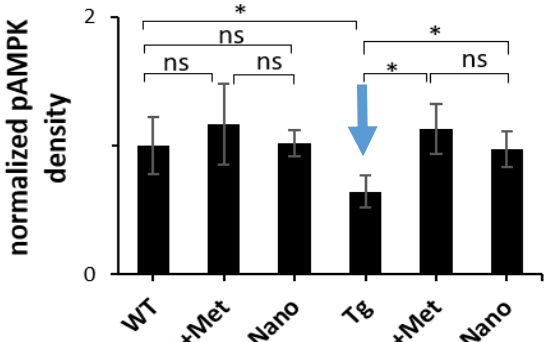


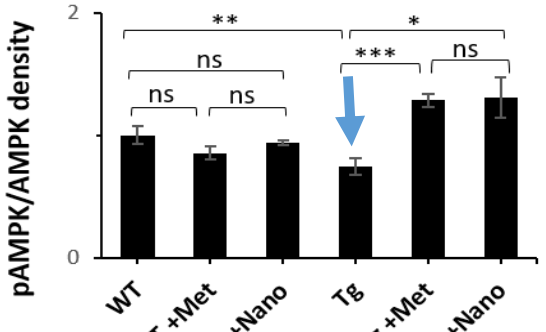




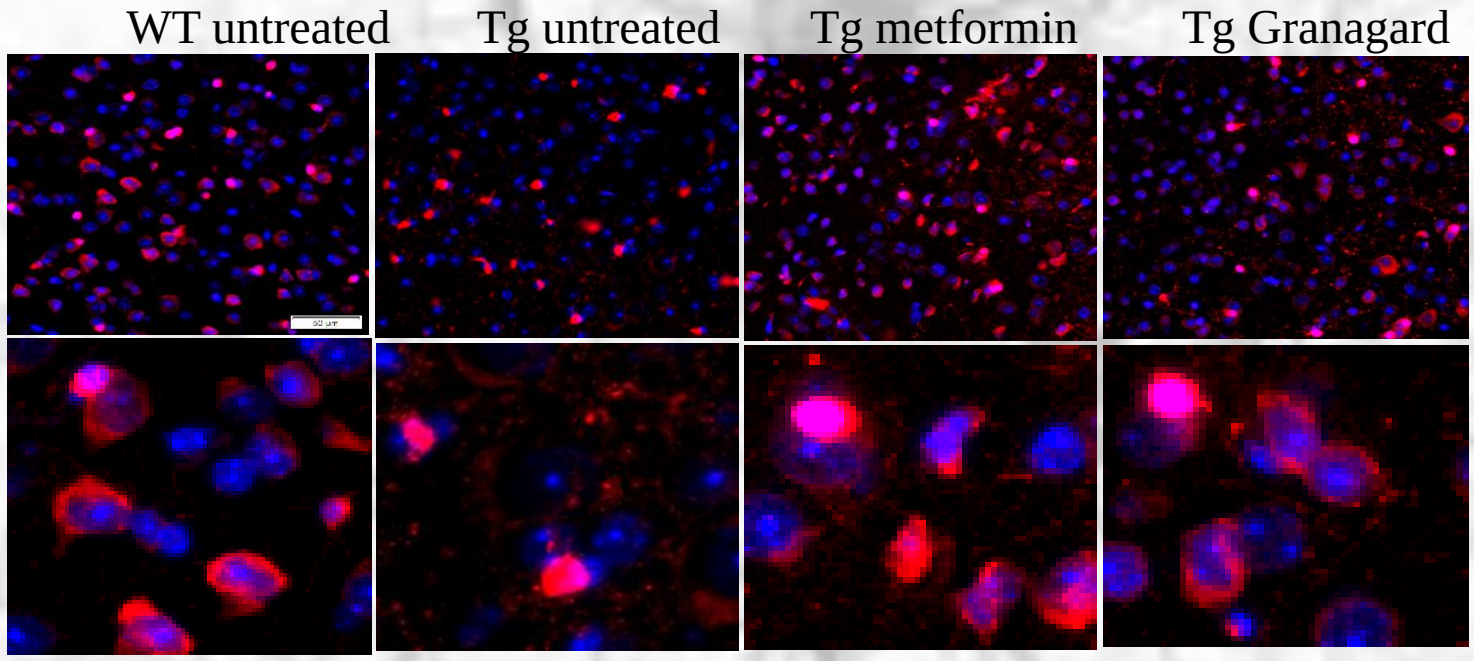


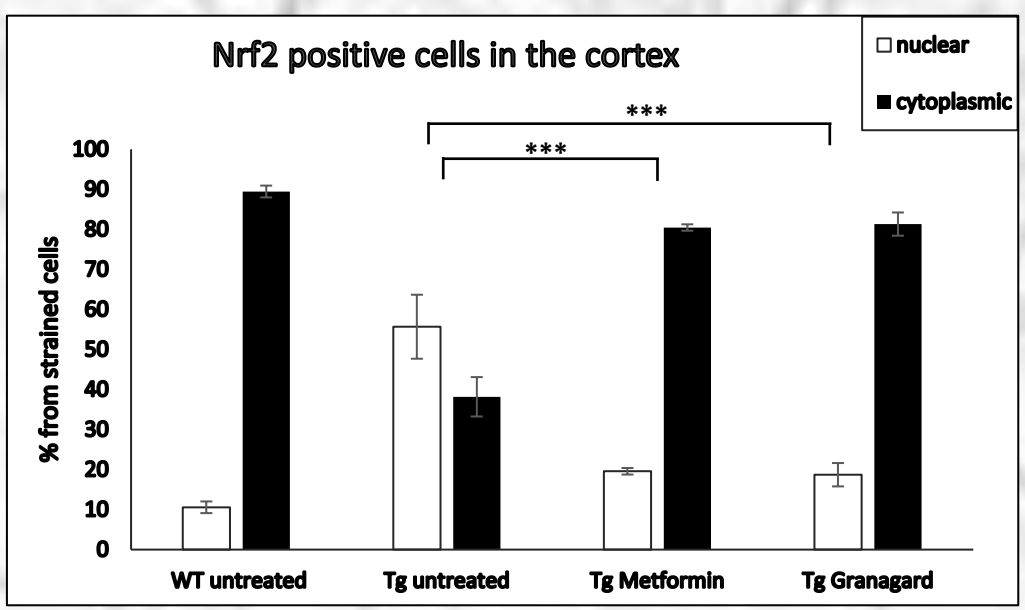


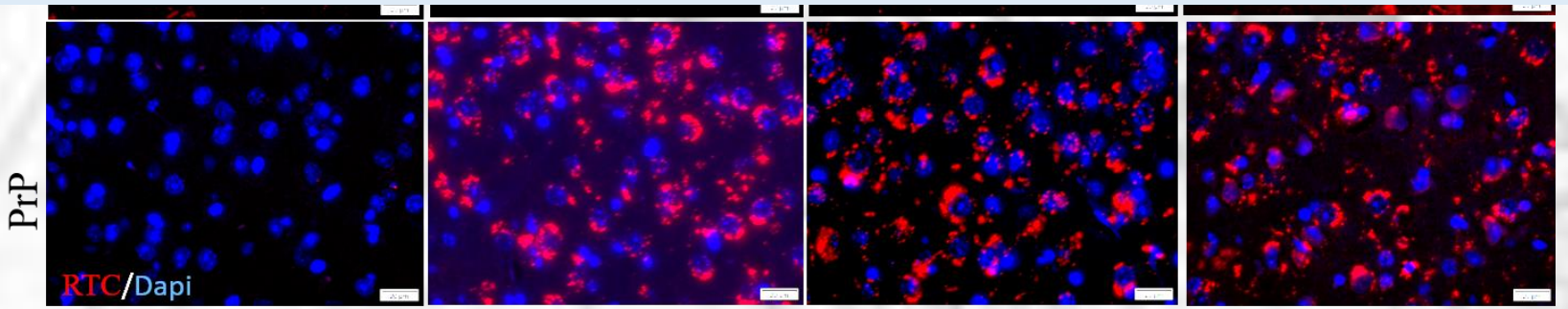


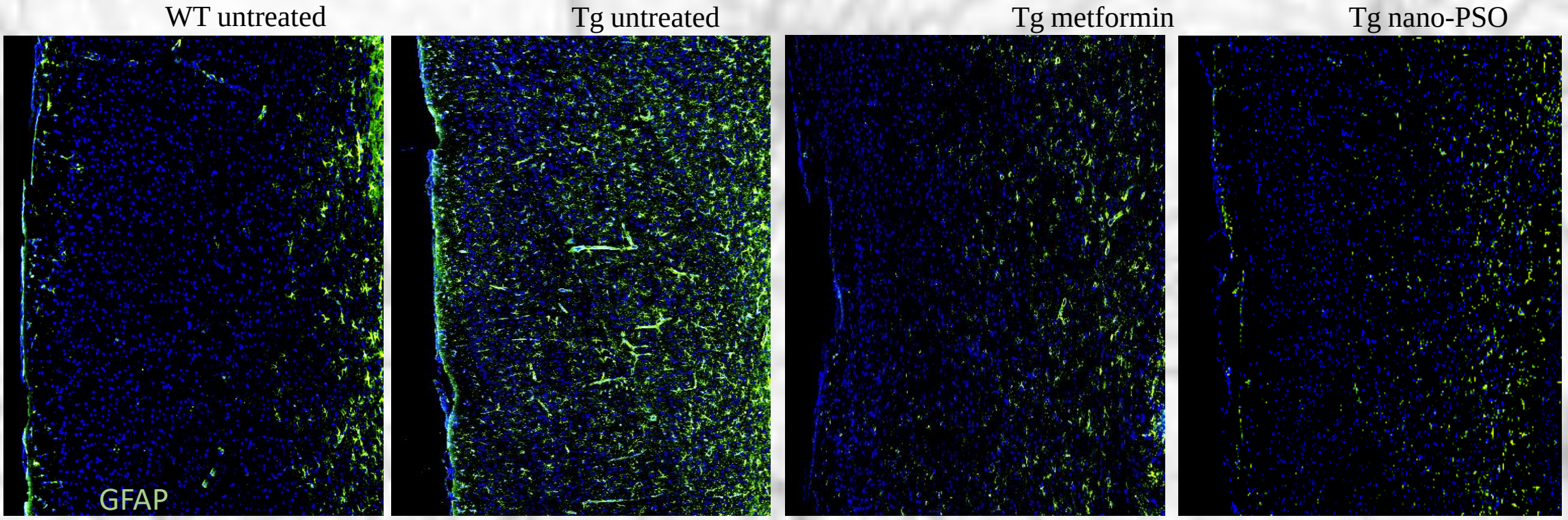


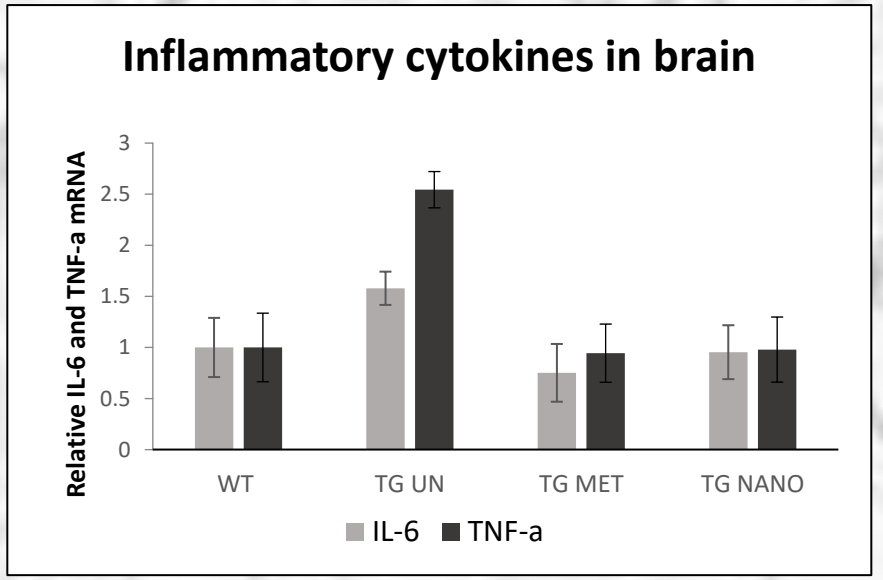
Granagard and Metformin induce the expression of Nrf2 in brains of Tg E199K mice






PrP
RTC/Dapi



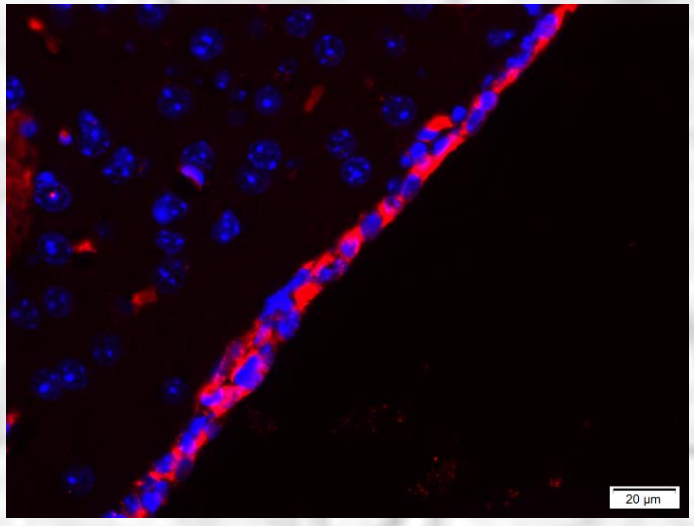


Inhibition of the inflammatory response by Metformin and Granagard

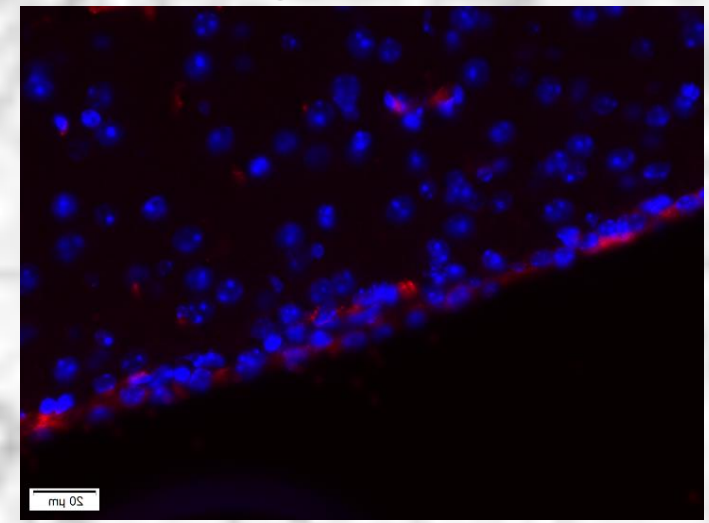
Aging and Neurodegeneration reduce the levels of stem cells in the brain

Metformin and Granagard both increased the levels of endogenous stem cells in Tg brain

WT

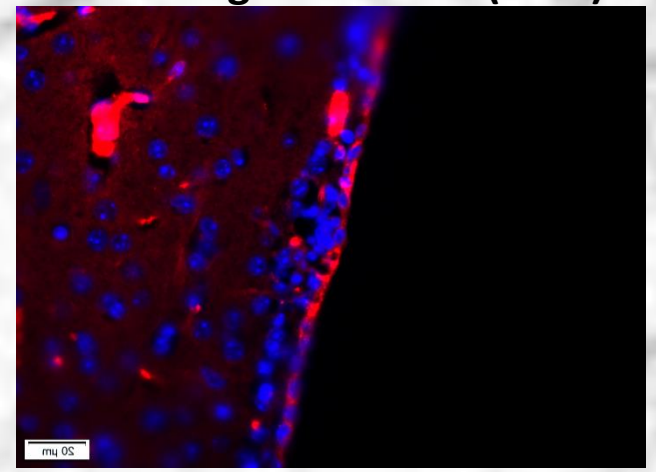


Tg untreated

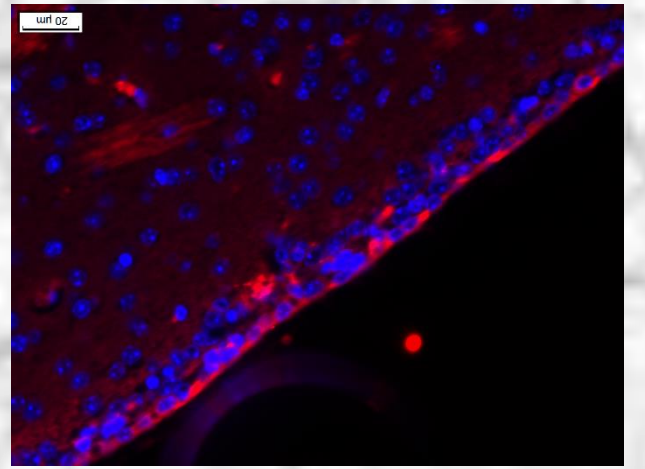


Nestin
Dapi

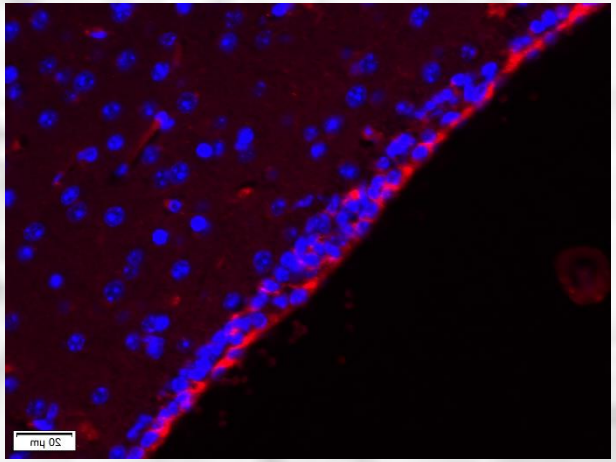
Tg metformin (4MT)



Tg Granagard(1.5 MT)



Tg Granagard (continues)



Neurodegeneration

Will Granagard work here?
yes

Will Metformin work here?

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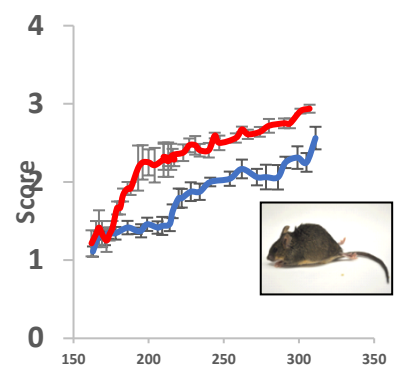
↓pAMPK
↑IGF-1
↓cox4-1
↓Hif-1
↓NRF-2
↓Stem-Cells production
↓Inflammatory response

Disease advance

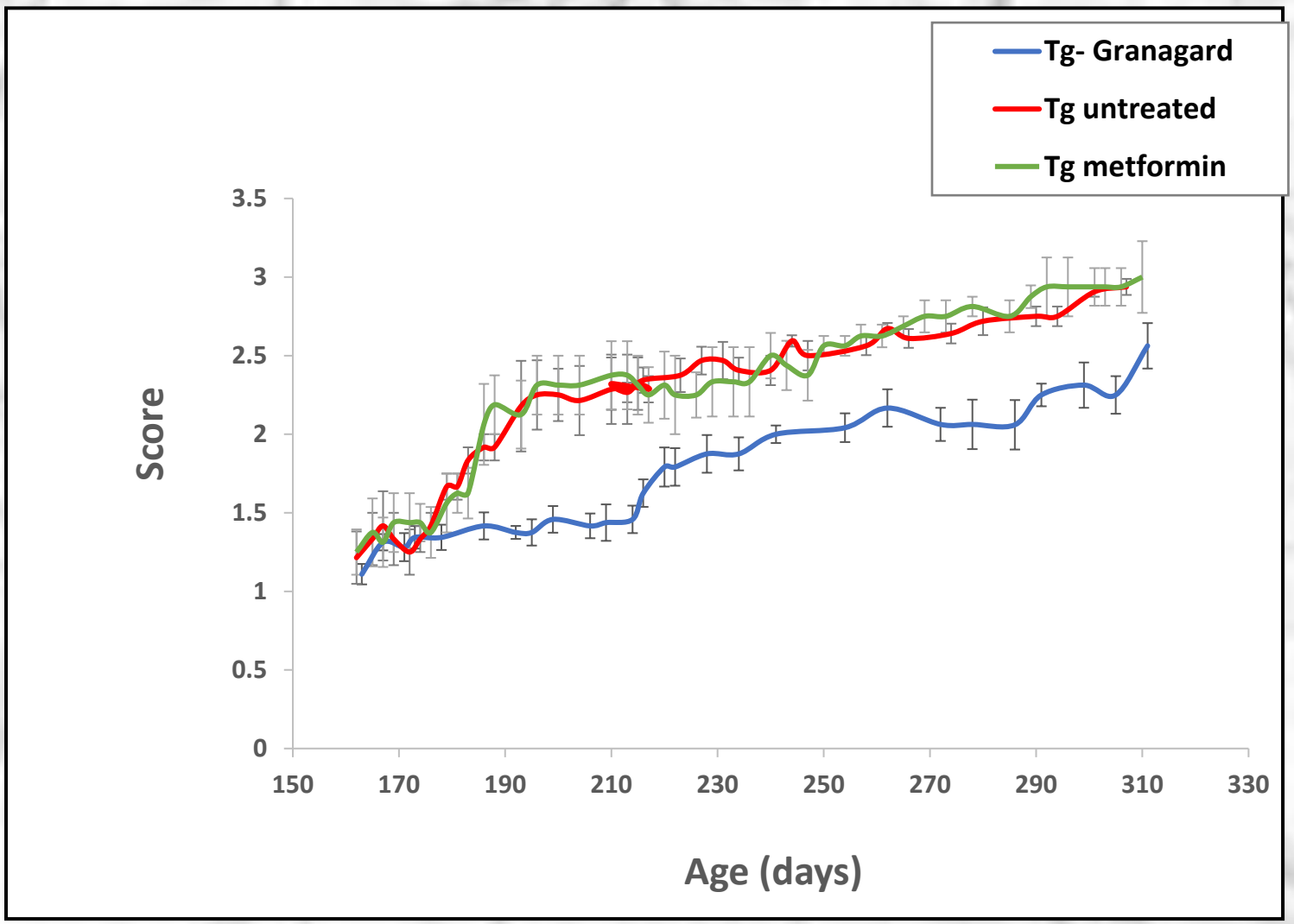
Key Protein Accumulation
PrP ?

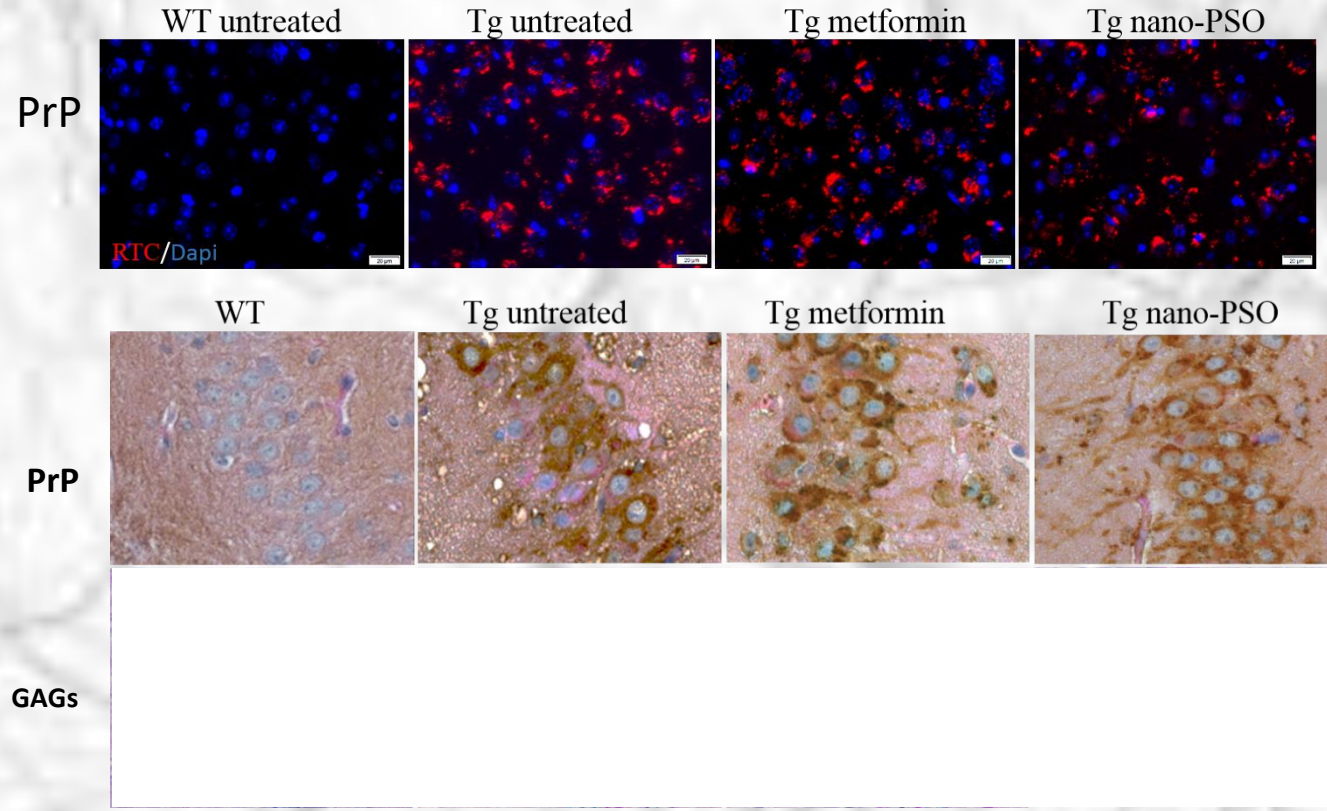
GAGs accumulation
Brains, urine?

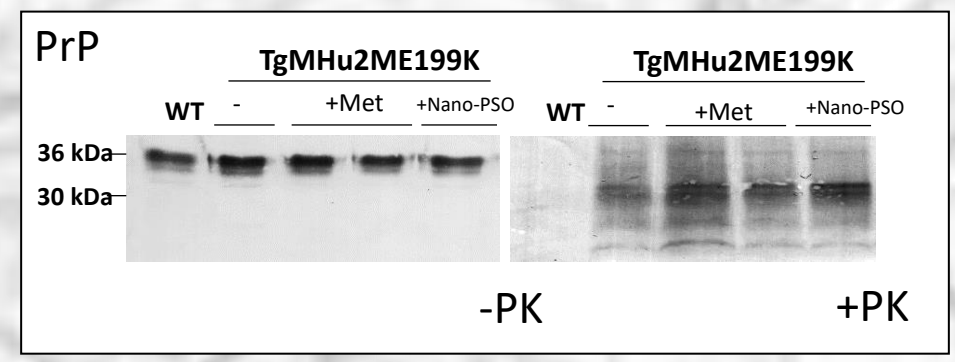
Lysosomal enzymes expression?

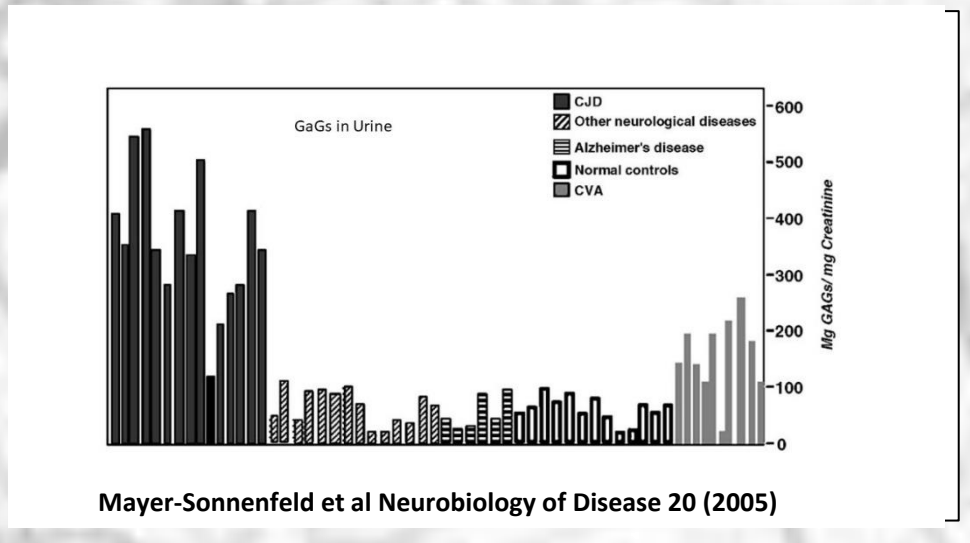


Metformin administration had no clinical effect on disease advance of TgMHu2ME199K mice

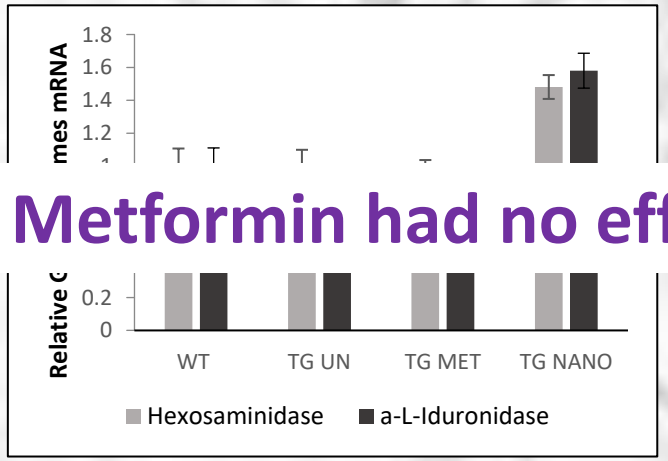








Mayer-Sonnenfeld et al Neurobiology of Disease 20 (2005)



Metformin had no effect on disease specific CJD markers

Comparing anti-aging hallmark activities of Metformin and Nano-PSO in a mouse model of genetic Creutzfeldt-Jakob Disease

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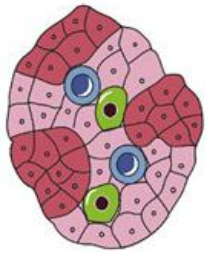
AMPK
Prion

ABSTRACT

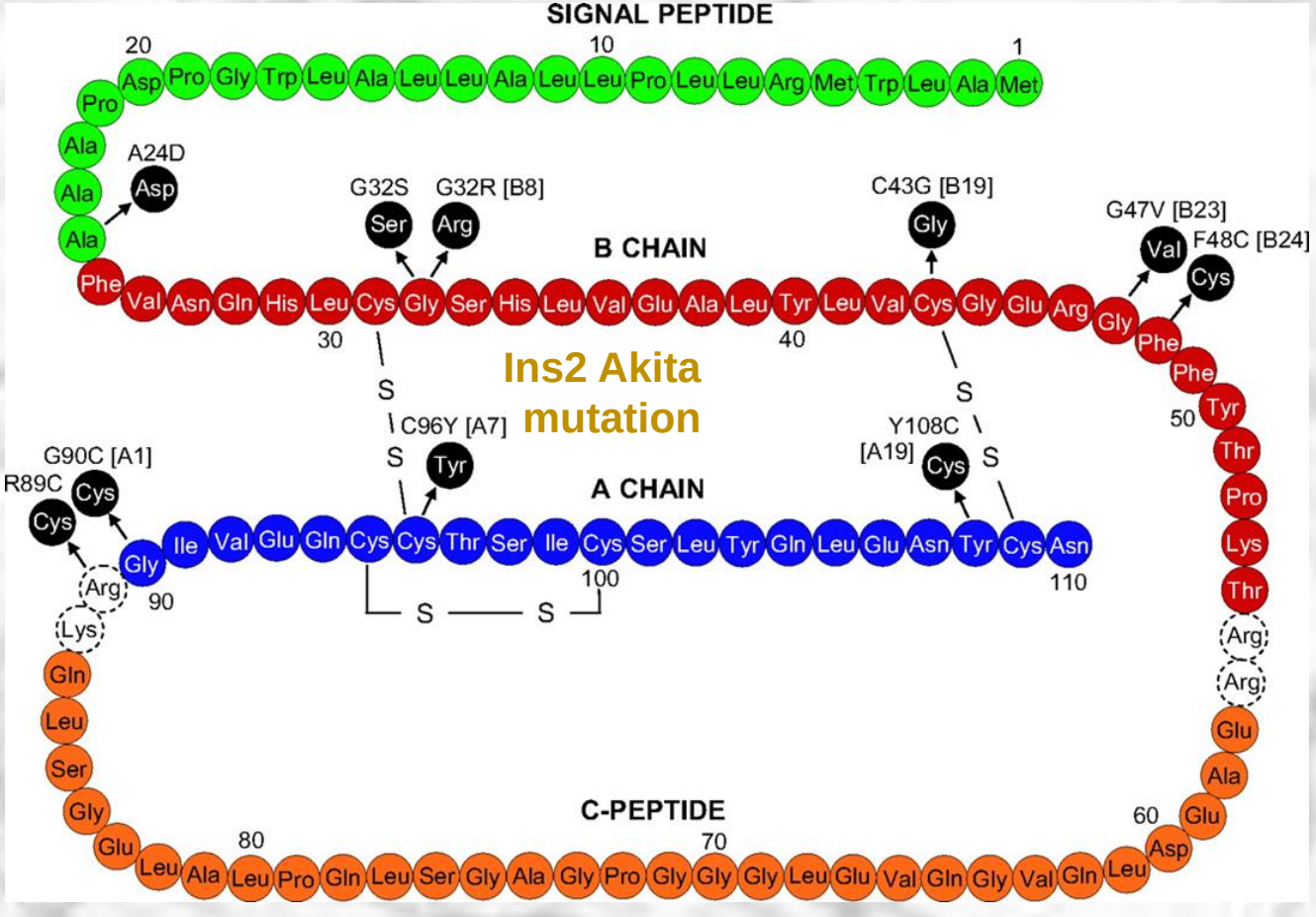
Advanced age is the main risk factor for the manifestation of late onset neurodegenerative diseases. Metformin, an anti-diabetic drug, was shown to extend longevity, and to ameliorate the activity of recognized aging hallmarks. Here, we compared the clinical, pathologic and biochemical effects of Metformin to those of Nano-PSO (Granagard), a brain targeted anti-oxidant shown by us to delay disease advance in transgenic mice mimicking for genetic Creutzfeldt Jacob disease (CJD) linked to the E200KPrP mutation. We demonstrate that both Metformin and Nano-PSO reduced aging hallmarks activities such as activated AMPK, the main energy sensor of cells as well as Nrf2 and COX IV1, regulators of oxidation, and mitochondrial activity. Both compounds reduced inflammation and increased stem cells production, however did not decrease PrP accumulation. As opposed to Nano-PSO, Metformin neither delayed clinical disease advance in these mice nor reduced the accumulation of sulfated glycosaminoglycans, a pathologic feature of prion disease. We conclude that elevation of anti-aging markers may not be sufficient to delay the fatal advance of genetic CJD.

The “Akita” model of type 1 diabetes

Akita mice

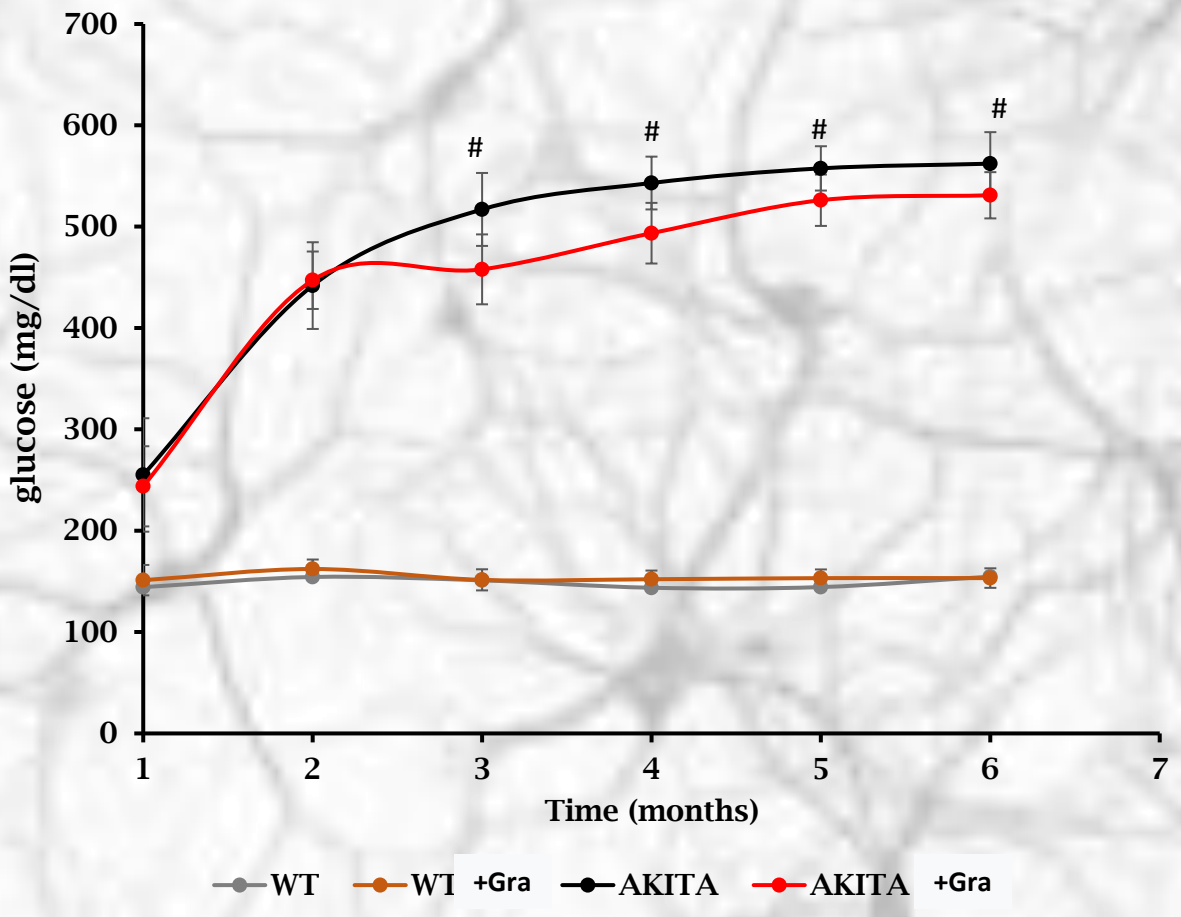


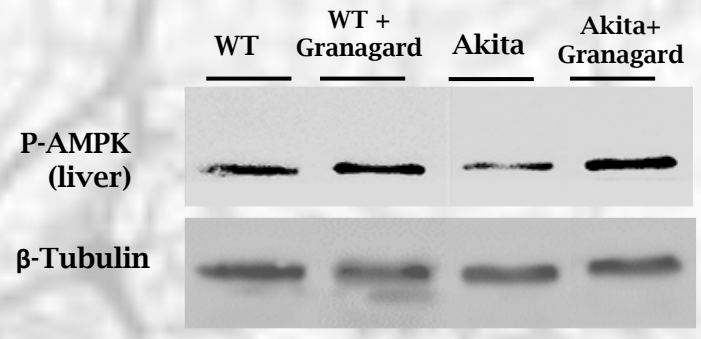
ER stress
B-cell dysfunction
Insulin deficiency

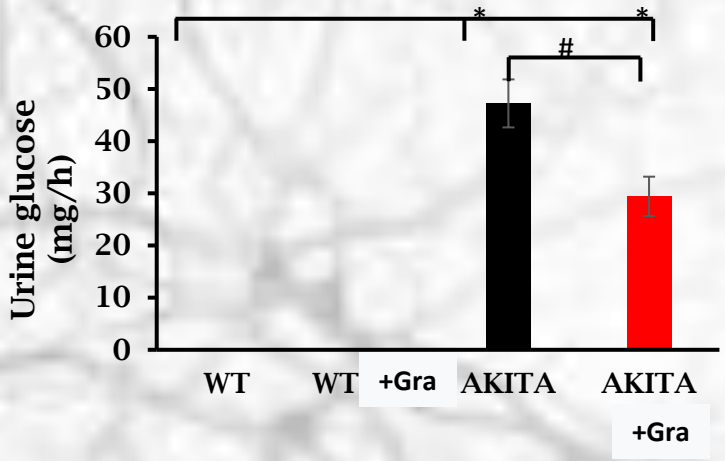


Mutant Insulin aggregates and mimics Type 1 diabetes

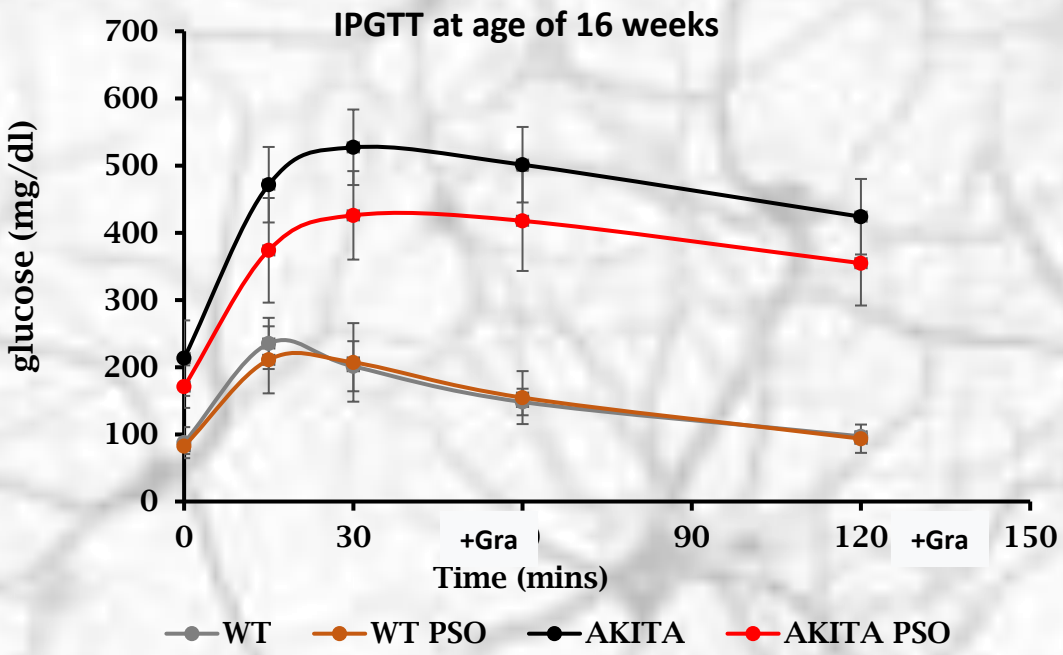
Granagard administration reduces glucose levels in blood and urine of Akita mice

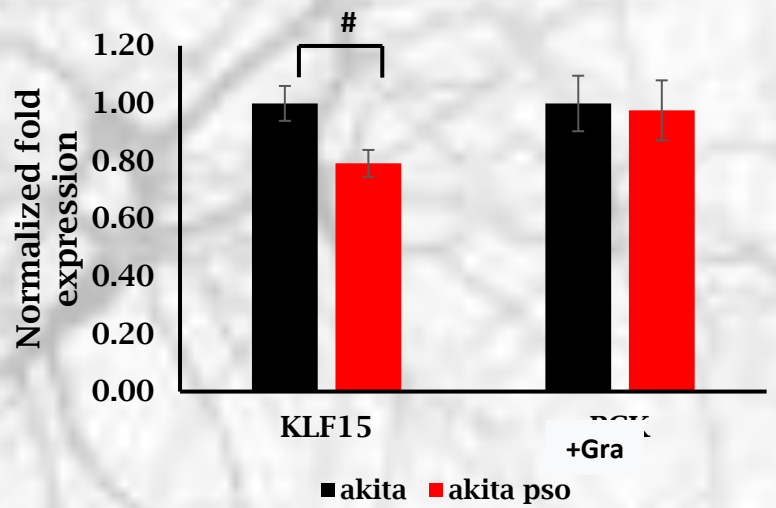


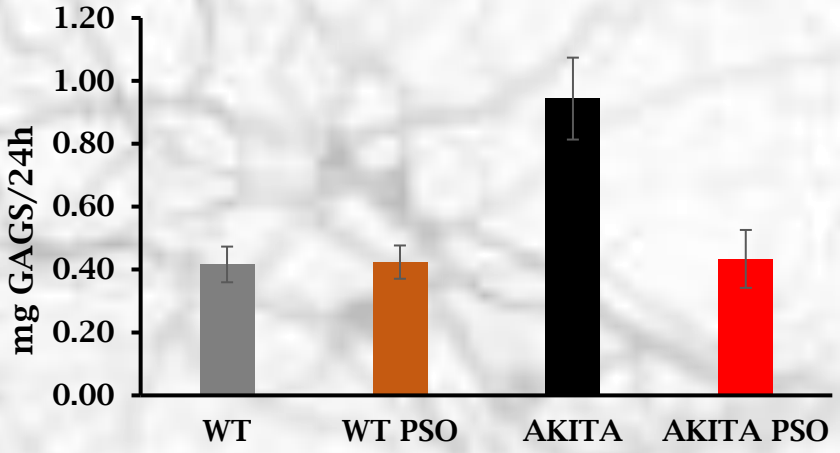




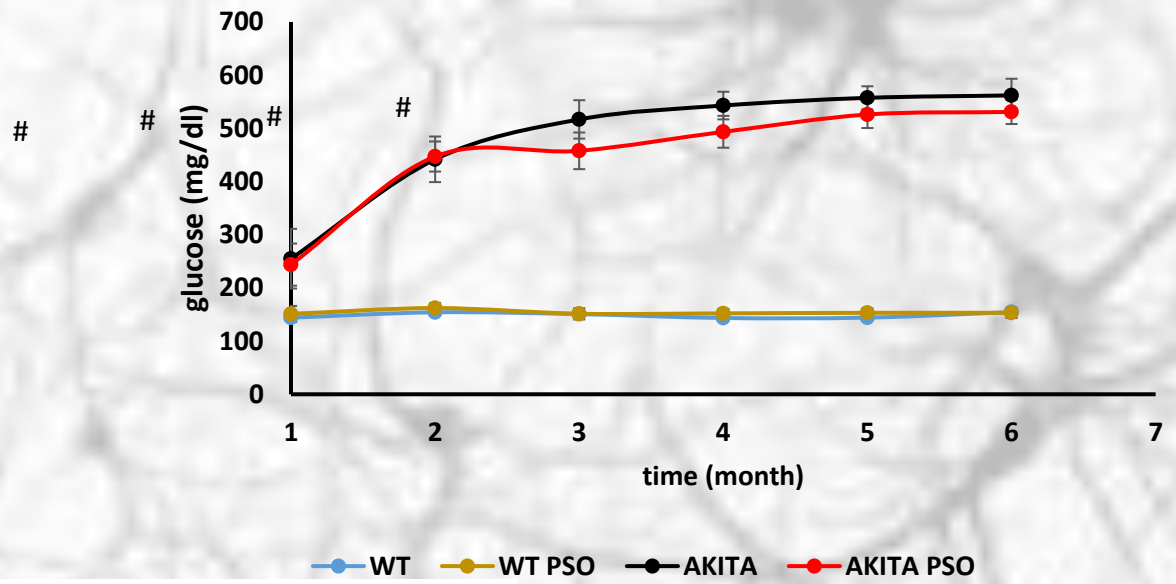
Granagrad reduced blood glucose through inhibition of gluconeogenesis enzymes activity and reduce insulin resistance

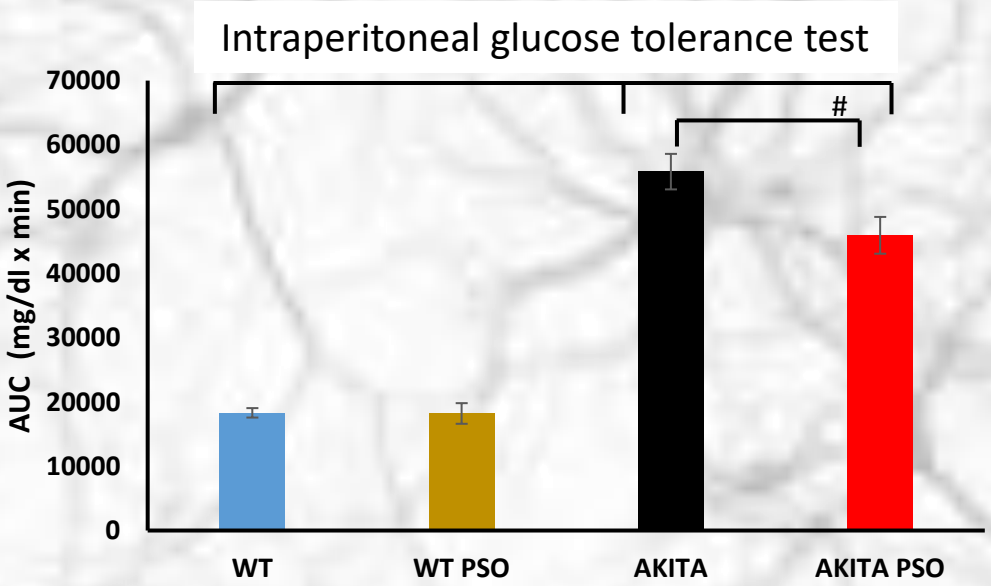


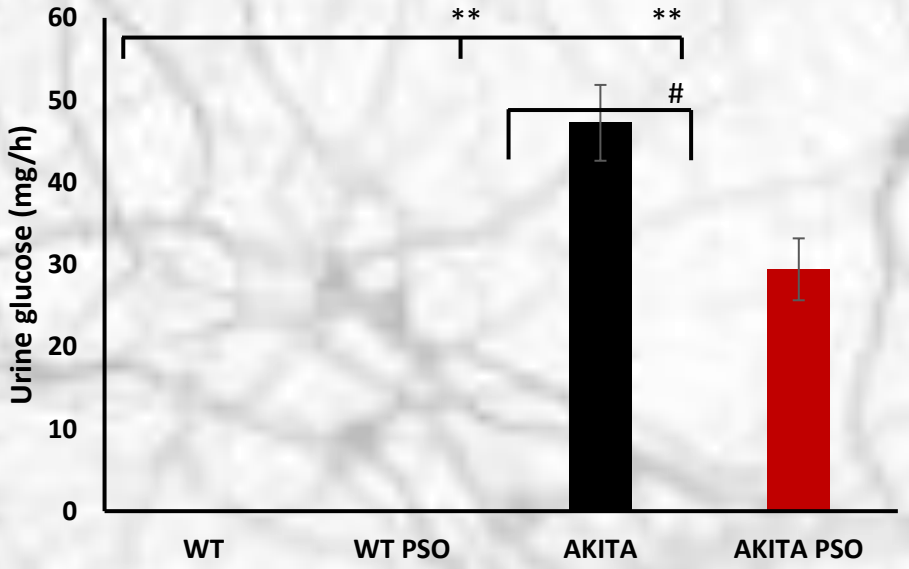




Granagard reduced fasting blood glucose and glucose tolerance on Akita mice







Conclusions

Aging and Neurodegeneration? It is complicated

Anti aging drugs may delay onset of neurodegeneration
or Anti aging drugs may reveal more neurodegenerative cases

The common factors between these mechanism are
ENERGY ACTIVATION, OXIDATIVE STRESS AND HYPOXIA

These factors should be stabilized so brain cells can live as long as possible

Misfolded protein aggregates and brain GAGs accumulation are specific for neurodegeneration

The anti oxidant mechanism of anti diabetic drugs may help increase cells survival

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Prof Tamir Ben Hur/ Dr Nina Fainstein (neurodegeneration)
Prof Ann Saada (mitochondria)
Prof Dimitri Karussis/ Dr Panayota Petrou (MS clinical trial)
Rambam Memory Clinic (MCI trial)
Prof Hana Rosenmann/ Dr Keren Nitzan (5FAD mice project)
Prof Hagai Pick Tel Aviv University Traumatic brain injury
Prof Esther Kahana and the Ichilov CJD clinic (CJD families)

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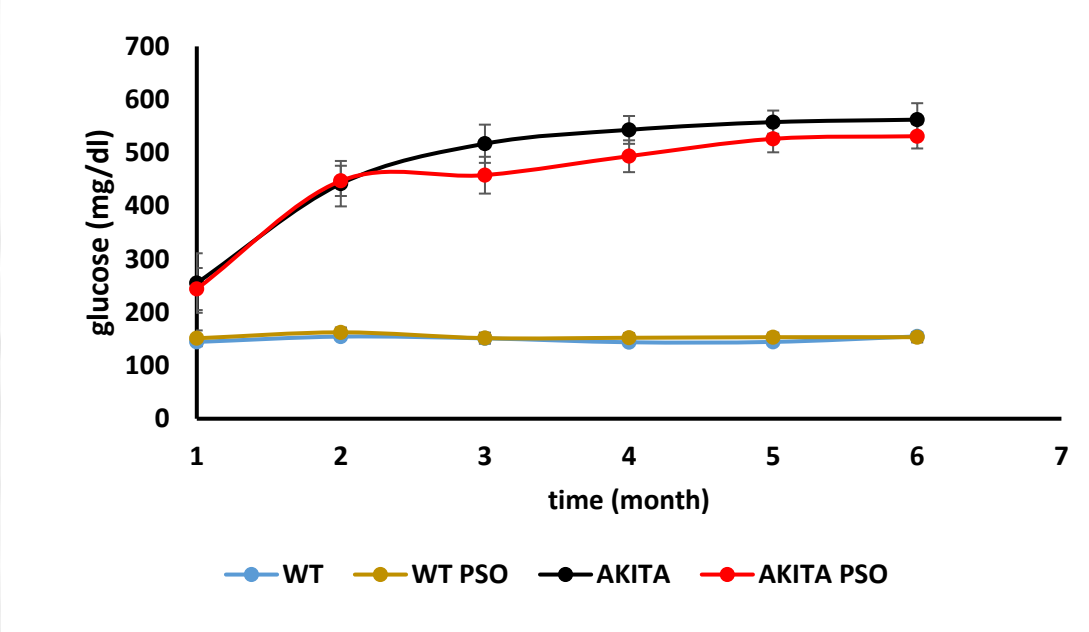
Supherb
The Granalix Team

Granalix

Granagard reduced fasting blood glucose and glucose tolerance on Akita mice



From Guy Keller PhD Thesis, in collaboration with Profs Ann Saada and Gil Leibovitz laboratory