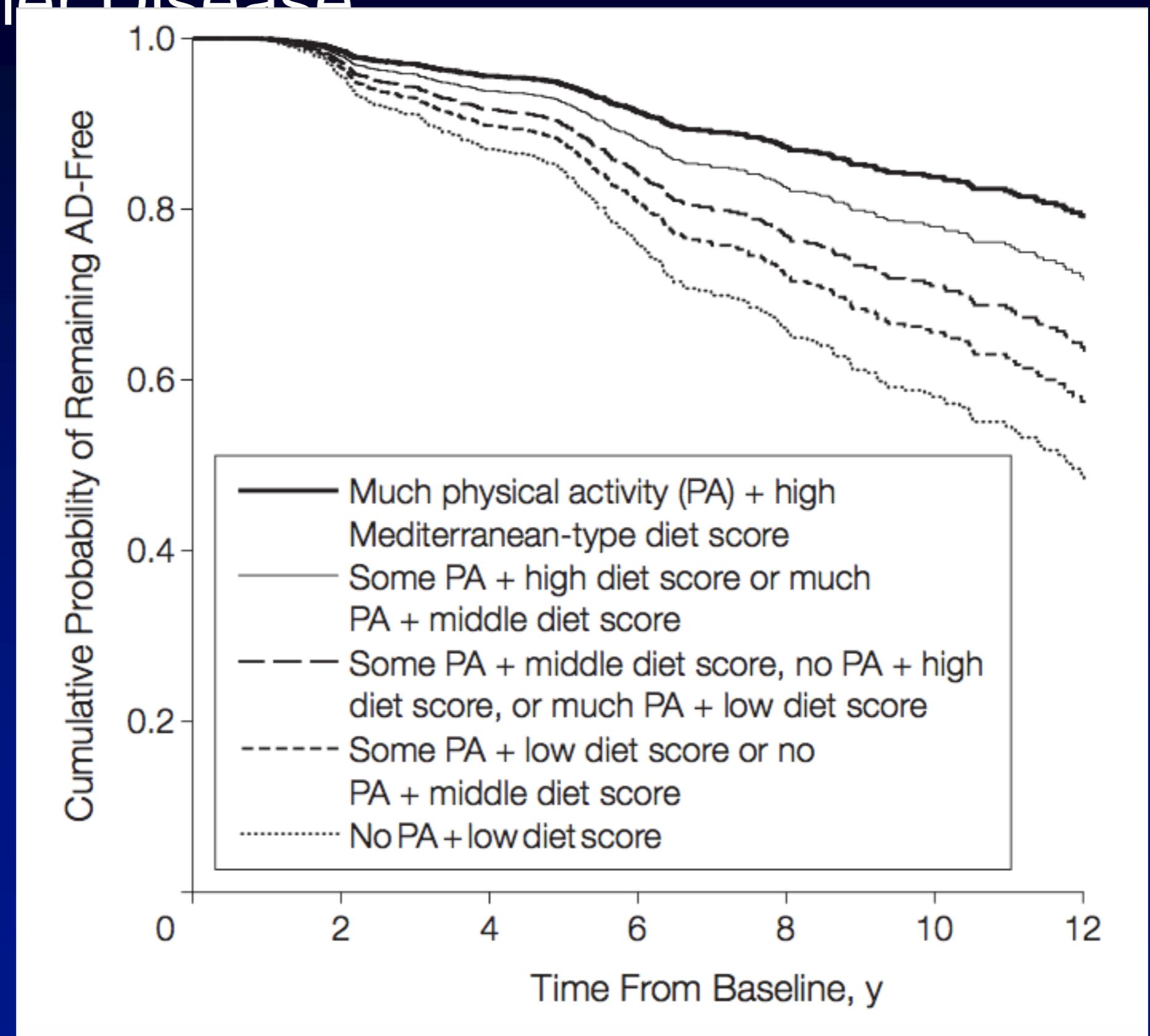


# Leveraging Lifestyle for Brain Health

David Perlmutter, MD

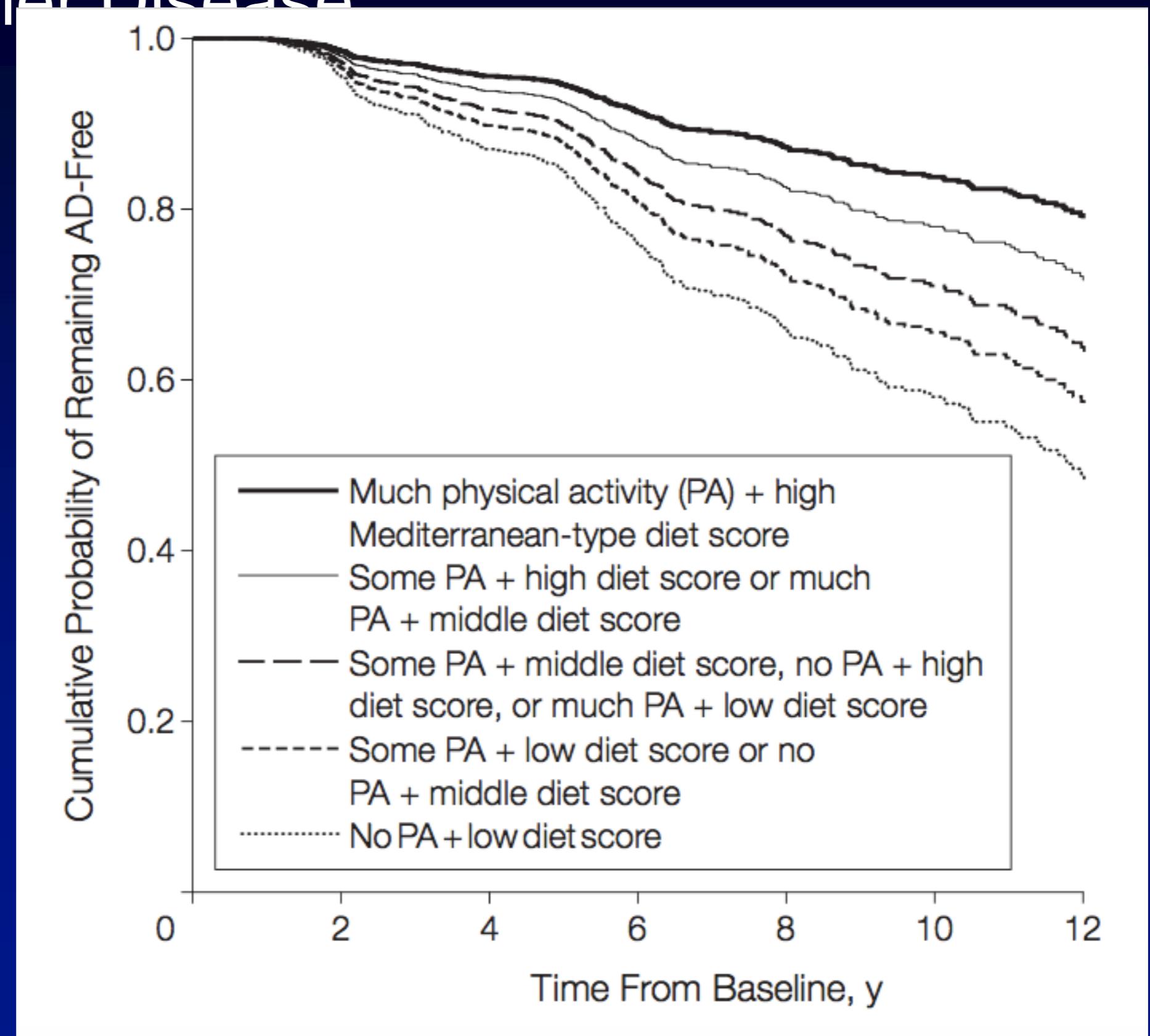
# Physical Activity, Diet, and Risk of Alzheimer Disease



# Physical Activity, Diet, and Risk of Alzheimer Disease

- 1880 community living elderly - dementia free
- Diet and physical activity measurements
- Neurological and neuropsychological exams every 1.5 years, 1992-2006

# Physical Activity, Diet, and Risk of Alzheimer Disease



Apolipoprotein E4 is associated with improved cognitive function with a high parasite burden

If the E4 allele is so strongly associated with cardiovascular disease, accelerated cognitive decline in aging, and Alzheimer's disease, why is it so prevalent worldwide?

Apolipoprotein E4 is associated with improved cognitive function with a high parasite burden

Why is the E4 allele even more prevalent in tropical areas with high parasitic burdens?

# Tsimane - Bolivian Amazon

- 15,000 individuals.
- Small-scale horticulture, hunting, fishing,gathering.
- No access to sanitation, electricity or running water.



# Tsimane - Bolivian Amazon

- Over 2/3 of adults have active helminth infections.
- 24% carry at least one E4 allele.
- None carry the E2 allele.



# Apolipoprotein E4 is associated with improved cognitive function with a high parasite burden

- 372 subjects from 28 villages
- APOE genotyping
- ESR, eosinophil count (parasitic load)
- 7-part cognitive battery

# Apolipoprotein E4 is associated with improved cognitive function with a high parasite burden

- E3/E3 - 76.1%
- E3/E4 - 21.3%
- E4/E4 - 2.6%

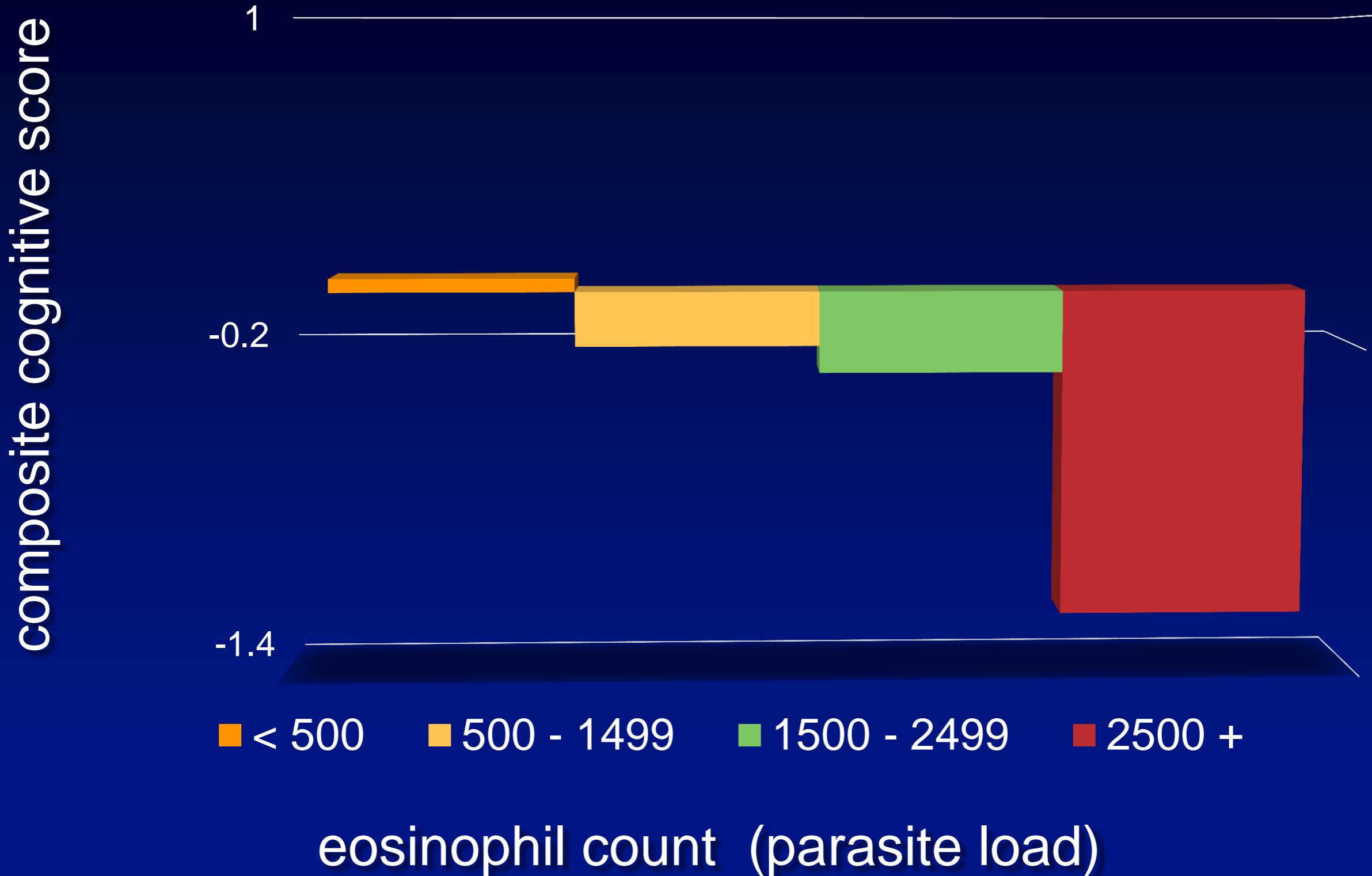
# Apolipoprotein E4 is associated with improved cognitive function with a high parasite burden

- E2 - 0%
- 23.9% carry at least one copy of E4

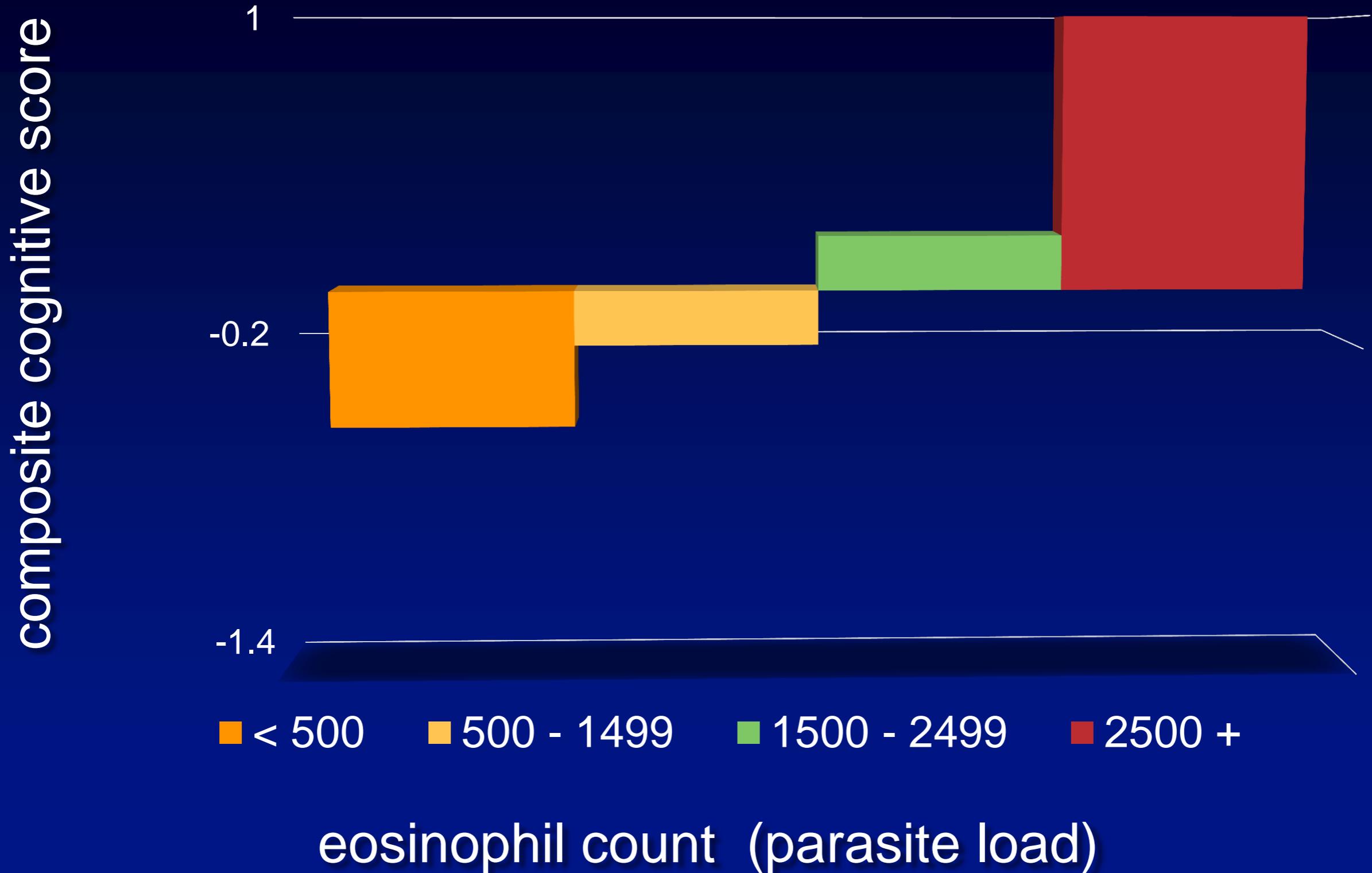
# Apolipoprotein E4 is associated with improved cognitive function with a high parasite burden

- E2 - 0%
- 23.9% carry at least one copy of E4

# APOE 3



# APOE 4



Apolipoprotein E4 is associated with improved cognitive function with a high parasite burden

For homozygous E3/E3 carriers, higher eosinophil counts are associated with poorer performance on all cognitive measures.

# Apolipoprotein E4 is associated with improved cognitive function with a high parasite burden

E4 carriers also showed significantly lower eosinophil counts, suggesting potentially protective effects of E4 against parasitic infection or burden. This result implies that E4 mitigates the effects of pathogen burden through at least 2 routes: by lowering the parasite load itself and by reducing its deleterious effects

# Apolipoprotein E4 is associated with improved cognitive function with a high parasite burden

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# Hygiene and the world distribution of Alzheimer's Disease

Molly Fox<sup>1,\*</sup>, Leslie A. Knapp<sup>1,2</sup>, Paul W. Andrews<sup>3</sup> and Corey L. Fincher<sup>4</sup>

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## Abstract

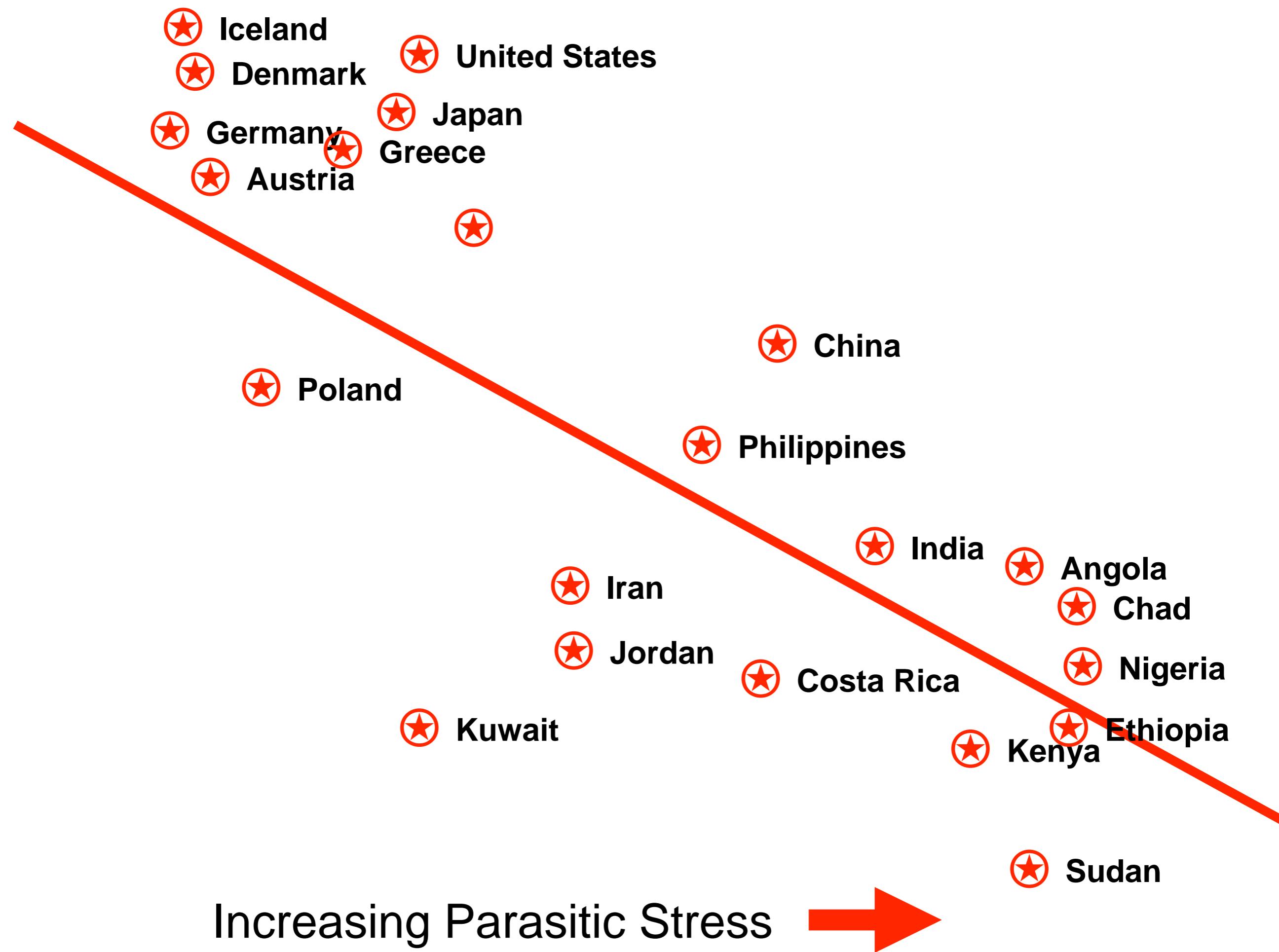
**Background and objectives:** Alzheimer's Disease (AD) shares certain etiological features with autoimmunity. Prevalence of autoimmunity varies between populations in accordance with variation in environmental microbial diversity. Exposure to microorganisms may improve individuals' immunoregulation in ways that protect against autoimmunity, and we suggest this may also be the case for AD. Here we investigate whether differences in microbial diversity can explain patterns of age-adjusted AD rates between countries.

rates between countries.

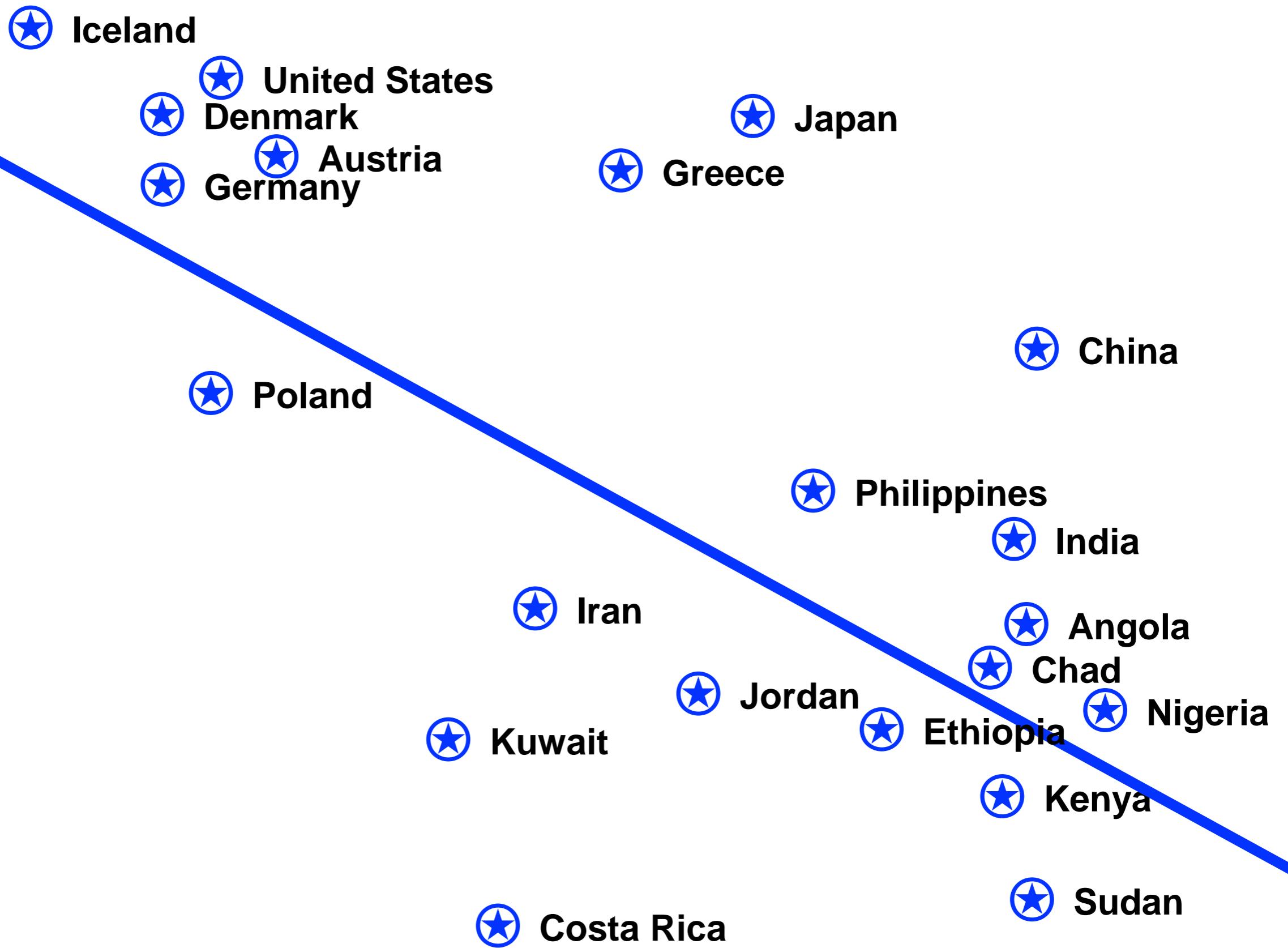
differences in microbial diversity can explain patterns of age-adjusted AD suggests this may also be the case for AD. Here we investigate whether

# Hygiene and the world distribution of Alzheimer's Disease

- Epidemiological evidence for a relationship between microbial environment and age-adjusted disease burden
- Comparison to hygiene (parasite load) with Alzheimer's incidence

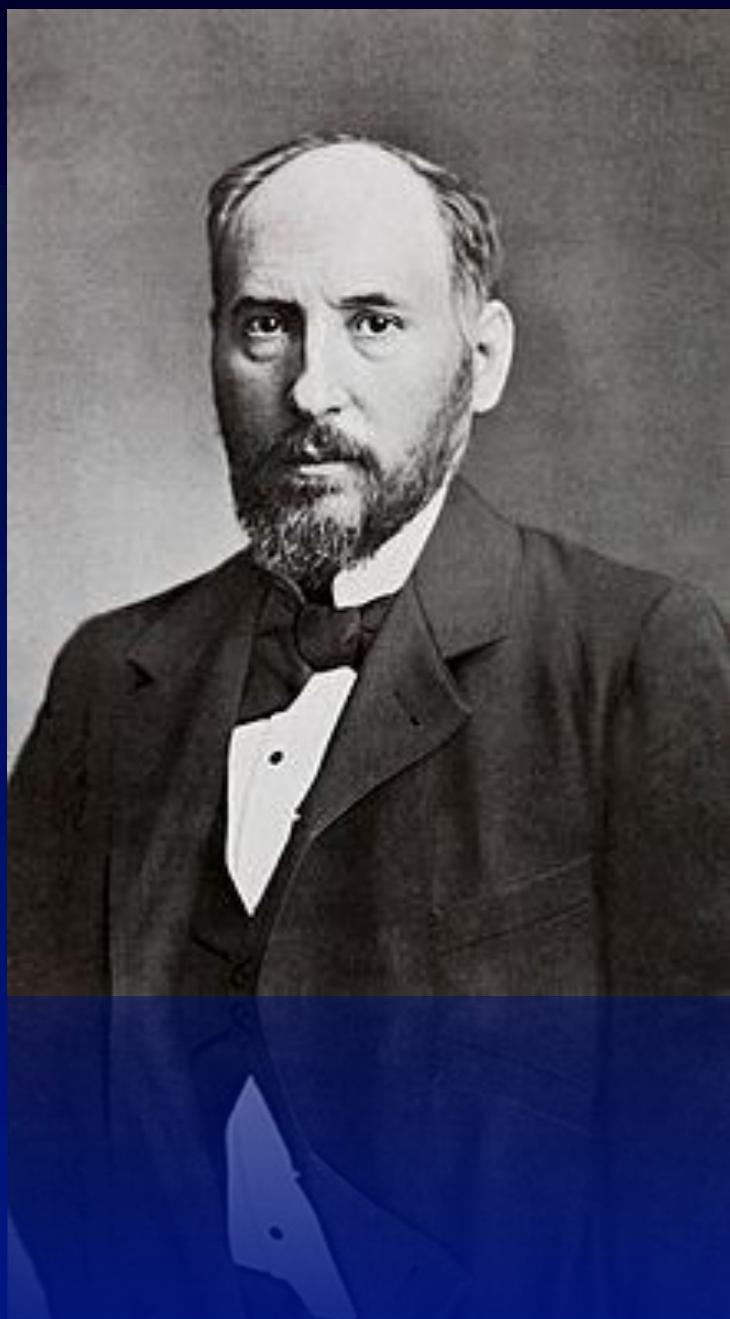


Increasing Alzheimer's Prevalence ↑



# Hygiene and the world distribution of Alzheimer's Disease

**Conclusions:** Variation in hygiene may partly explain global patterns in AD rates. Microorganism exposure may be inversely related to AD risk. These results may help predict AD burden in developing countries where microbial diversity is rapidly diminishing.



“In the adult centers, the nerve paths are something fixed, and immutable: everything must die, nothing may be regenerated.”

- Santiago Ramón y Cajal, Nobel laureate  
(1852-1934)



“Our study demonstrates that cell genesis occurs in human brains and that the human brain retains the potential for self-renewal throughout life.”

- Dr. Peter Eriksson, 1998

# Neurogenesis in the adult human hippocampus

PETER S. ERIKSSON<sup>1</sup>, EKATERINA PERELIEVA<sup>1</sup>, THOMAS BJÖRK-ERIKSSON<sup>2</sup>, ANN-MARIE ALBORN<sup>1</sup>, CLAES NORDBORG<sup>3</sup>, DANIEL A. PETERSON<sup>3</sup> & FRED H. GAGE<sup>1</sup>

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<sup>2</sup>Laboratory of Genetics, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, California 92037, USA

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The genesis of new cells, including neurons, in the adult human brain has not yet been demonstrated. This study was undertaken to investigate whether neurogenesis occurs in the adult human brain, in regions previously identified as neurogenic in adult rodents and monkeys. Human brain tissue was obtained postmortem from patients who had been treated with the thymidine analog, bromodeoxyuridine (BrdU), that labels DNA during the S phase. Using immunofluorescent labeling for BrdU and for one of the neuronal markers, NeuN, calbindin or neuron specific enolase (NSE), we demonstrate that new neurons, as defined by these markers, are generated from dividing progenitor cells in the dentate gyrus of adult humans. Our results further indicate that the human hippocampus retains its ability to generate neurons throughout life.

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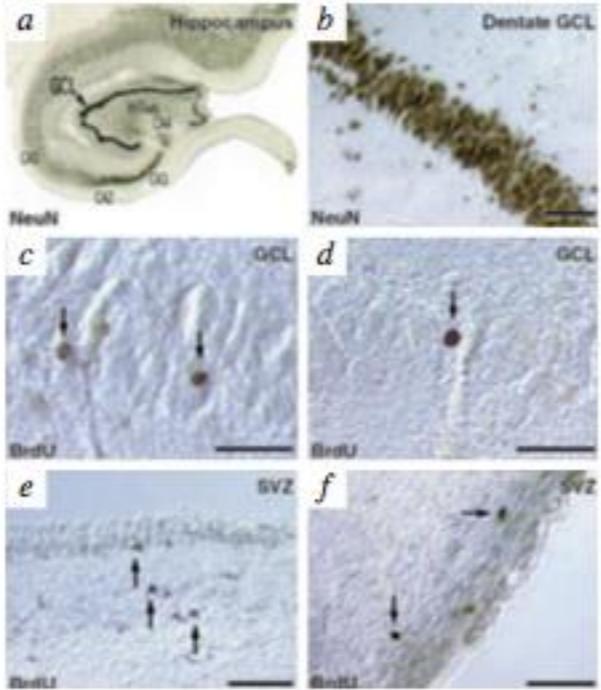
Loss of neurons is thought to be irreversible in the adult human brain, because dying neurons cannot be replaced. This inability to generate replacement cells is thought to be an important cause of neurological disease and impairment. In most brain regions, the generation of neurons is generally confined to a discrete developmental period. Exceptions are found in the dentate gyrus and the subventricular zone of several species that have been shown to generate new neurons well into the postnatal and adult period<sup>1–4</sup>. Granule neurons are generated throughout life from a population of continuously dividing progenitor cells residing in the subgranular zone of the dentate gyrus in the rodent brain<sup>5</sup>. 'Newborn' neurons generated from these progenitor cells migrate into the granule cell layer, differentiate, extend axons and express neuronal marker proteins<sup>1–6</sup>.

We examined whether progenitor cells reside in the adult human hippocampus and whether new neurons are born within the dentate gyrus of the adult human brain. Postmortem tissue from the hippocampus and the subventricular zone of caudate nucleus was obtained from cancer patients ( $n = 5$ ) who received

one intravenous infusion (250 mg; 2.5 mg/ml, 100 ml) of bromodeoxyuridine (BrdU) for diagnostic purposes<sup>7</sup>. One patient diagnosed with a similar type and location of cancer, but without BrdU treatment, was included as a control. A thymidine analog, BrdU is incorporated into the DNA of dividing cells and can be detected immunohistochemically in their progeny<sup>4,7–11</sup>.

## Cell genesis and survival in the adult human dentate gyrus

The number of surviving labeled, proliferating progenitors was

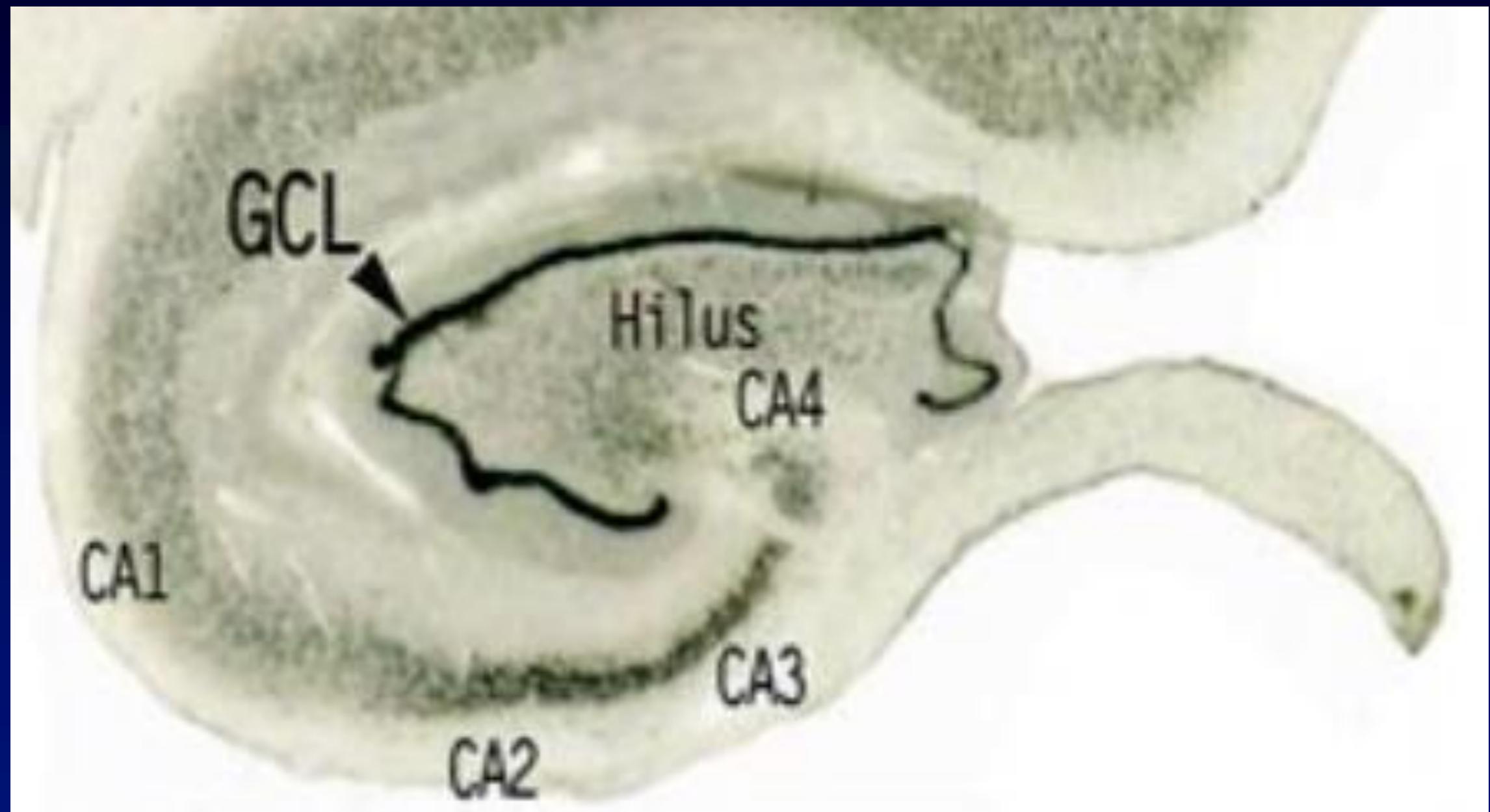


**Fig. 1** Newly generated cells can be detected in the adult human brain in patients previously treated with BrdU. *a*, The hippocampal region of the adult human brain immunoperoxidase-stained for the neuronal marker NeuN. *b*, The hippocampal dentate gyrus granule cell layer (GCL) visualized with immunoperoxidase staining for NeuN. *c*, Differential interference contrast photomicrograph showing BrdU-labeled nuclei (arrows) in the dentate granule cell layer (GCL). *d*, Differential interference contrast photomicrograph showing a BrdU-labeled nucleus (arrow) in the human dentate GCL. BrdU-positive nuclei have a rounded appearance and resemble the chromatin structure of mature granule cells and are found within the granule cell layer. *e*, Differential interference contrast photomicrograph showing BrdU-positive cells (arrows) adjacent to the ependymal lining in the subventricular zone of the human caudate nucleus. Cells with elongated nuclei resembling migrating cells are in the rat subventricular zone (SVZ). *f*, Differential interference contrast photomicrograph showing BrdU-positive cells (arrows) with round to elongated nuclei in the subventricular zone of the human caudate nucleus. All scale bars represent 50  $\mu$ m.

# Neurogenesis in the adult human hippocampus

First demonstration of neurogenesis in humans

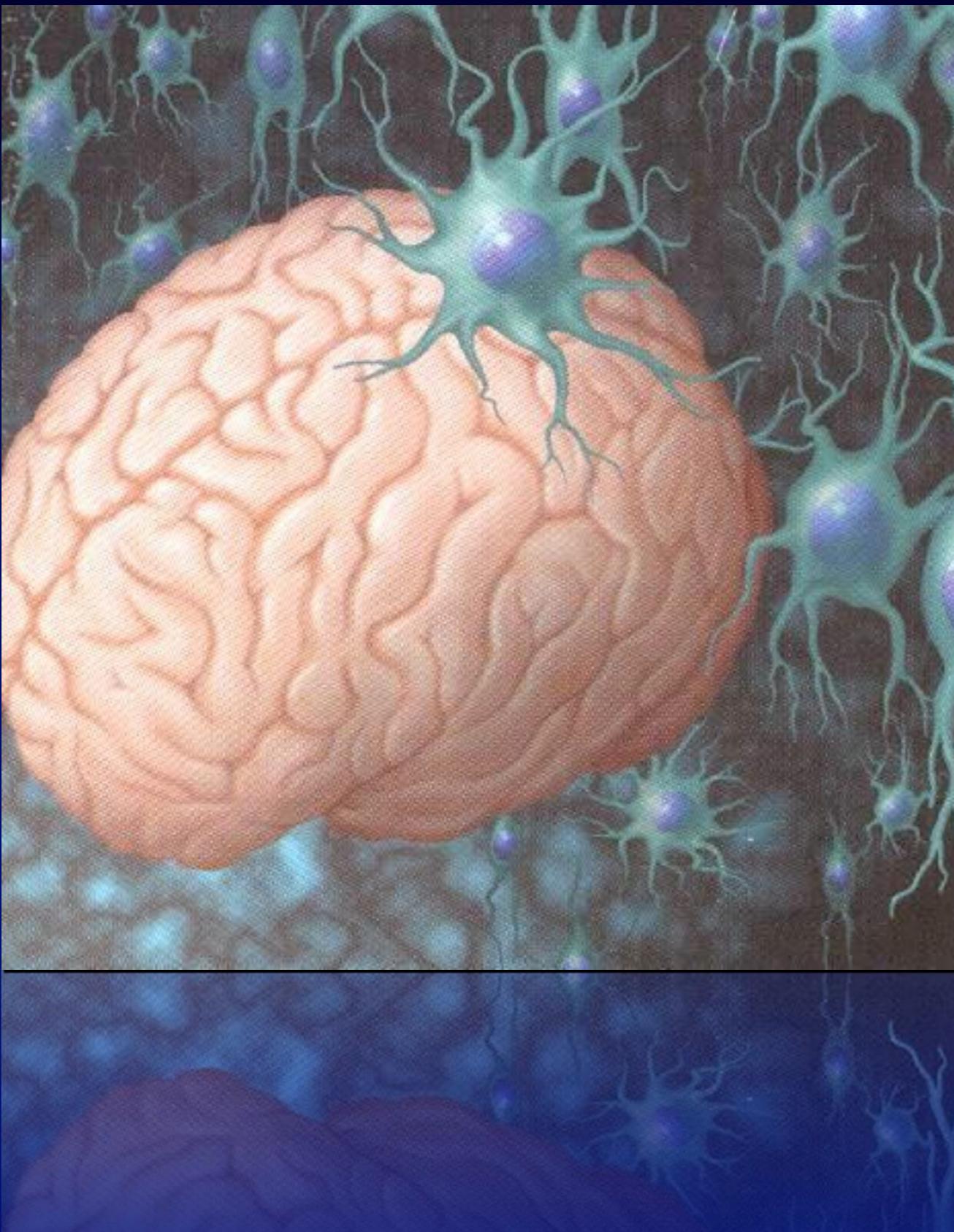
# Neurogenesis in the adult human hippocampus



# Neurogenesis in the adult human hippocampus

“Our study demonstrates that cell genesis occurs in human brains and that the human brain retains the potential for self-renewal throughout life.”

# Neurotrophic Factors



- Nerve Growth Factor (NGF)
- Brain Derived Neurotrophic Factor (BDNF)
- Basic Fibroblast Growth Factor (bFGF)

**BDNF**

**Neurogenesis**

**Neuroplasticity**

**Neuronal differentiation**

**Neuronal Repair**

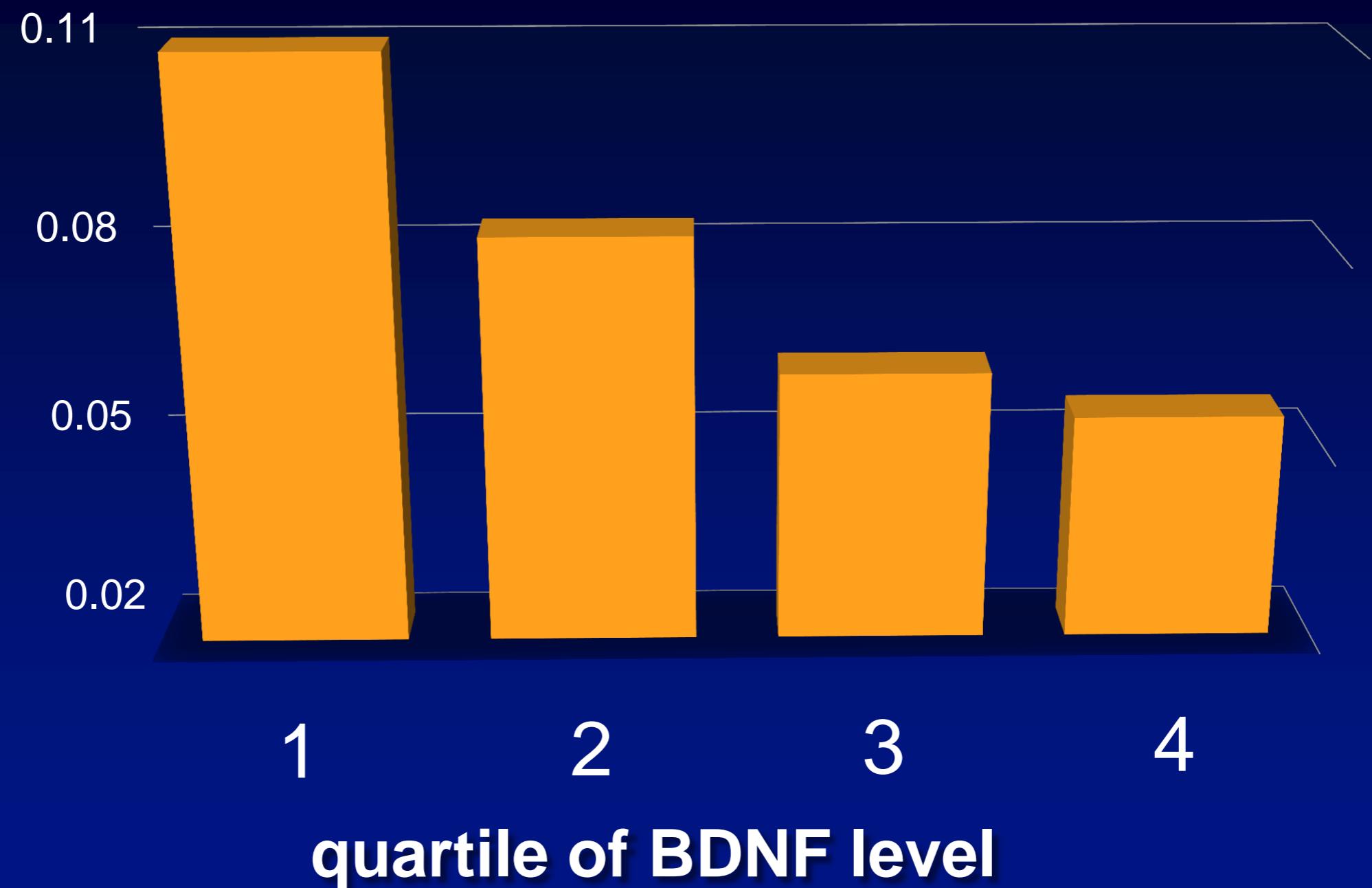
**Synaptogenesis**

# Serum Brain-Derived Neurotrophic Factor and the Risk for Dementia

## The Framingham Heart Study

- 2131 dementia free subjects
- Age  $\geq$  60 years
- BDNF at baseline
- Development of dementia
- Followed  $\sim$  10 years

dementia risk over 10 years



**quartile of BDNF level**

## Higher brain BDNF gene expression is associated with slower cognitive decline in older adults

- 535 older adults, annual cognitive assessment (mean 6.3 years)
- Autopsy - BDNF measured in prefrontal cortex
- Comparison of BDNF with cognitive decline
- Does Alzheimer's disease pathology/cognitive decline vary with BDNF expression?

# Higher brain BDNF gene expression is associated with slower cognitive decline in older adults

- Higher brain BDNF expression was associated with slower cognitive decline
- Cognitive decline was about 50% slower with the 90th percentile BDNF expression vs 10th

# What enhances BDNF?

- Exercise
- Turmeric
- Whole Coffee Fruit
- DHA
- Alpha lipoic acid
- Ketosis ( $\beta$ -hydroxybutyrate)

# Exercise training increases size of hippocampus and improves memory

## Exercise training increases size of hippocampus and improves memory

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Edited by Fred Gage, Salk Institute, San Diego, CA, and approved December 30, 2010 (received for review October 23, 2010)

The hippocampus shrinks in late adulthood, leading to impaired memory and increased risk for dementia. Hippocampal and medial temporal lobe volumes are larger in higher-fit adults, and physical activity training increases hippocampal perfusion, but the extent to which aerobic exercise training can modify hippocampal volume in late adulthood remains unknown. Here we show, in a randomized controlled trial with 120 older adults, that aerobic exercise training increases the size of the anterior hippocampus, leading to improvements in spatial memory. Exercise training increased hippocampal volume by 2%, effectively reversing age-related loss in volume by 1 to 2 y. We also demonstrate that increased hippocampal volume is associated with greater serum levels of BDNF, a mediator of neurogenesis in the dentate gyrus. Hippocampal volume declined in the control group, but higher preintervention fitness partially attenuated the decline, suggesting that fitness protects against volume loss. Caudate nucleus and thalamus volumes were unaffected by the intervention. These theoretically important findings indicate that aerobic exercise training is effective at reversing hippocampal volume loss in late adulthood, which is accompanied by improved memory function.

aging | brain | cognition | plasticity | MRI

Deterioration of the hippocampus precedes and leads to memory impairment in late adulthood (1, 2). Strategies to fight hippocampal loss and protect against the development of memory impairment has become an important topic in recent years from both scientific and public health perspectives. Physical activity, such as aerobic exercise, has emerged as a promising low-cost treatment to improve neurocognitive function that is accessible to most adults and is not plagued by intolerable side effects often found with pharmaceutical treatments (3). Exercise enhances learning and improves retention, which is accompanied by increased cell proliferation and survival in the hippocampus of rodents (4–6); effects that are mediated, in part, by increased production and secretion of BDNF and its receptor tyrosine kinase trkB (7, 8).

Aerobic exercise training increases gray and white matter volume in the prefrontal cortex (9) of older adults and increases the functioning of key nodes in the executive control network (10, 11). Greater amounts of physical activity are associated with sparing of prefrontal and temporal brain regions over a 9-y period, which reduces the risk for cognitive impairment (12). Further, hippocampal and medial temporal lobe volumes are larger in higher-fit older adults (13, 14), and larger hippocampal volumes mediate improvements in spatial memory (13). Exercise training increases cerebral blood volume (15) and perfusion of the hippocampus (16), but the extent to which exercise can modify the size of the hippocampus in late adulthood remains unknown.

To evaluate whether exercise training increases the size of the hippocampus and improves spatial memory, we designed a single-blind, randomized controlled trial in which adults were randomly

assigned to receive either moderate-intensity aerobic exercise 3 d/wk or stretching and toning exercises that served as a control. We predicted that 1 y of moderate-intensity exercise would increase the size of the hippocampus and that change in hippocampal volume would be associated with increased serum BDNF and improved memory function.

### Results

**Aerobic Exercise Training Selectively Increases Hippocampal Volume.** One hundred twenty older adults without dementia (Table 1) were randomly assigned to an aerobic exercise group ( $n = 60$ ) or to a stretching control group ( $n = 60$ ). Magnetic resonance images were collected before the intervention, after 6 mo, and again after the completion of the program. The groups did not differ at baseline in hippocampal volume or attendance rates (Table 2 and *SI Results*). We found that the exercise intervention was effective at increasing the size of the hippocampus. That is, the aerobic exercise group demonstrated an increase in volume of the left and right hippocampus by 2.12% and 1.97%, respectively, over the 1-y period, whereas the stretching control group displayed a 1.40% and 1.43% decline over this same interval (Fig. 1A). The moderating effect of aerobic exercise on hippocampal volume loss was confirmed by a significant Time  $\times$  Group interaction for both the left [ $F(2,114) = 8.25$ ;  $P < 0.001$ ;  $\eta_p^2 = 0.12$ ] and right [ $F(2,114) = 10.41$ ;  $P < 0.001$ ;  $\eta_p^2 = 0.15$ ] hippocampus (see Table 2 for all means and SDs).

As can be seen in Fig. 2, we found that aerobic exercise selectively increased the volume of the anterior hippocampus that included the dentate gyrus, where cell proliferation occurs (4, 6, 8), as well as subiculum and CA1 subfields, but had a minimal effect on the volume of the posterior section. Cells in the anterior hippocampus mediate acquisition of spatial memory (17) and show more age-related atrophy compared with the tail of the hippocampus (18, 19). The selective effect of aerobic exercise on the anterior hippocampus was confirmed by a significant Time  $\times$  Group  $\times$  Region interaction for both the left [ $F(2,114) = 4.05$ ;  $P < 0.02$ ;  $\eta_p^2 = 0.06$ ] and right [ $F(2,114) = 4.67$ ;  $P < 0.01$ ;  $\eta_p^2 = 0.07$ ] hippocampus. As revealed by *t* tests, the aerobic exercise group showed an increase in anterior hippocampus volume from baseline to after intervention [left:  $t(2,58) = 3.38$ ;  $P < 0.001$ ; right:  $t(2,58) = 4.33$ ;  $P < 0.001$ ] but demonstrated no change in the volume of the posterior hippocampus (both  $P > 0.10$ ). In contrast,

Author contributions: K.I.E., M.W.V., R.S.P., C.B., J.A.W., E. McAuley, and A.F.K. designed research; K.I.E., M.W.V., R.S.P., A.S., L.C., J.S.K., S.H., H.A., S.M.W., T.R.W., E. Mailey, V.J.V., S.A.M., B.D.P., E. McAuley, and A.F.K. performed research; K.I.E., M.W.V., and R.S.P. analyzed data; and K.I.E., M.W.V., R.S.P., and A.F.K. wrote the paper.

The authors declare no conflict of interest.

\*This Direct Submission article had a prearranged editor.

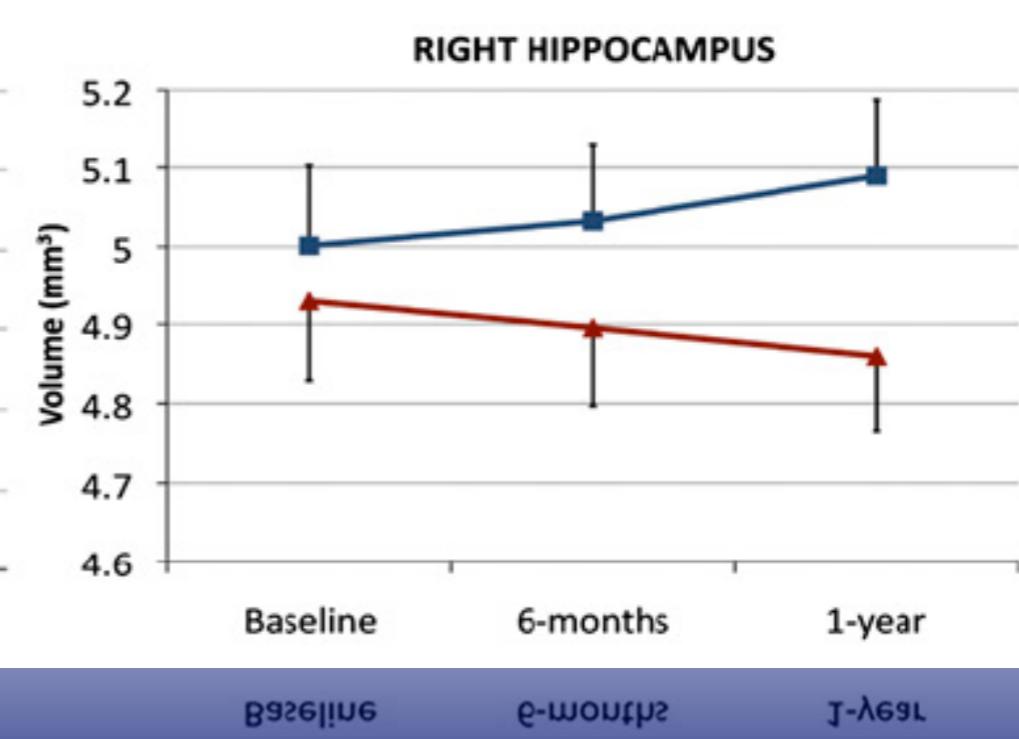
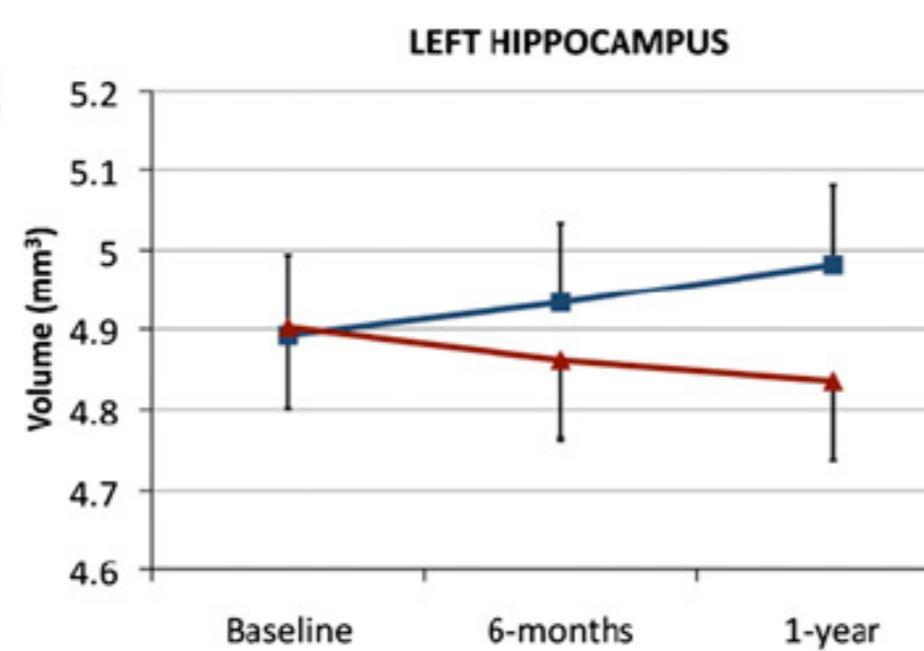
<sup>1</sup>To whom correspondence should be addressed. E-mail: a-kramer@illinois.edu.

This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1015950108/DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1015950108/DCSupplemental).

# Exercise training increases size of hippocampus and improves memory

- The hippocampus shrinks in late adulthood, leading to impaired memory and increased risk for dementia.
- Randomized controlled trial, 120 older adults, 1 year, stretching vs. aerobic exercise.
- Measurement of hippocampal volume, BDNF and memory function at baseline, 6 months and 1 year

## Hippocampus



# Exercise training increases size of hippocampus and improves memory

- Greater changes in serum BDNF were associated with greater increases in hippocampal volume

# Exercise training increases size of hippocampus and improves memory

“ These results clearly indicate that aerobic exercise is neuroprotective and that starting an exercise regimen later in life is not futile for either enhancing cognition or augmenting brain volume.”

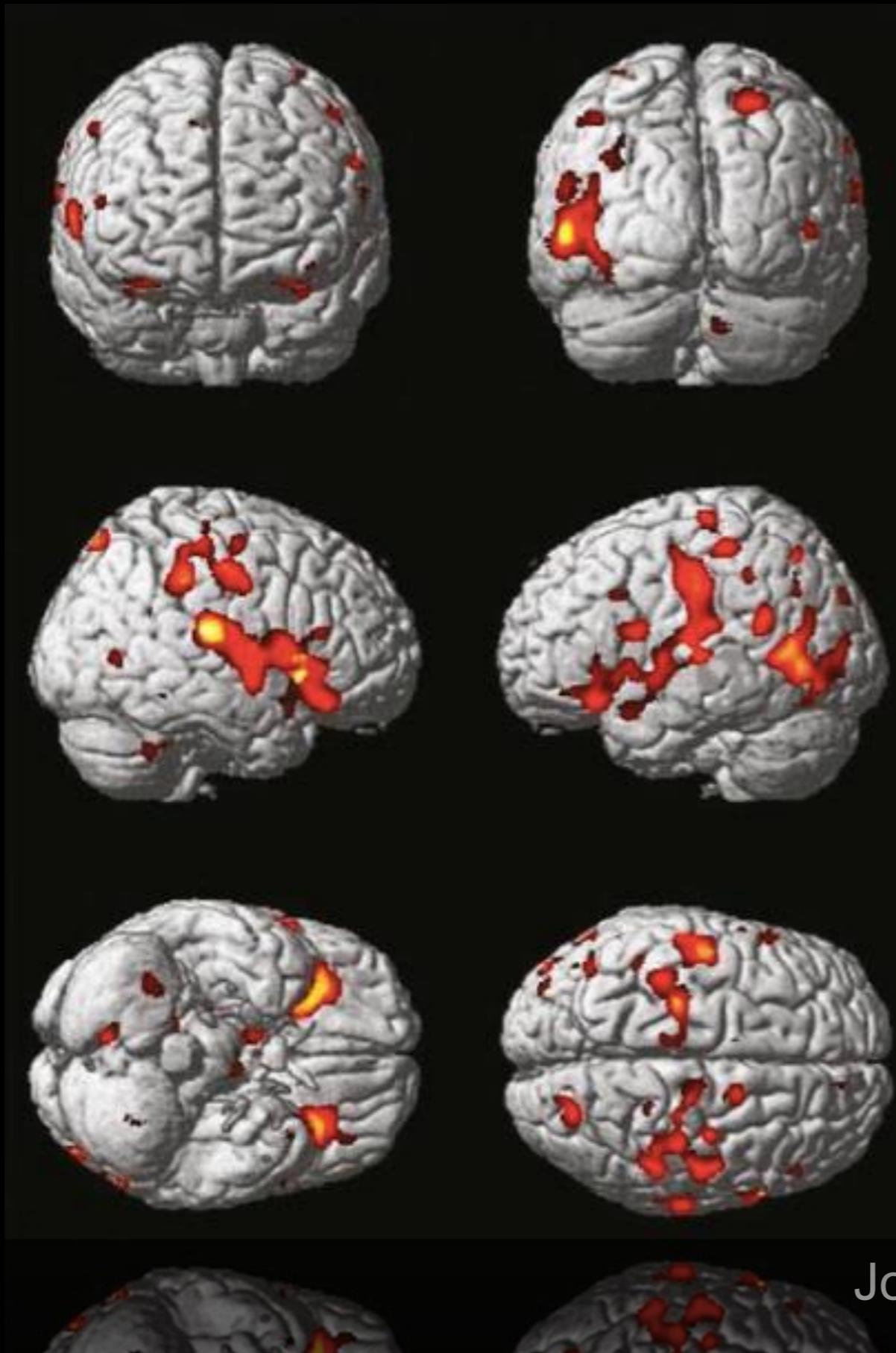
# Longitudinal Relationships between Caloric Expenditure and Gray Matter in the Cardiovascular Health Study

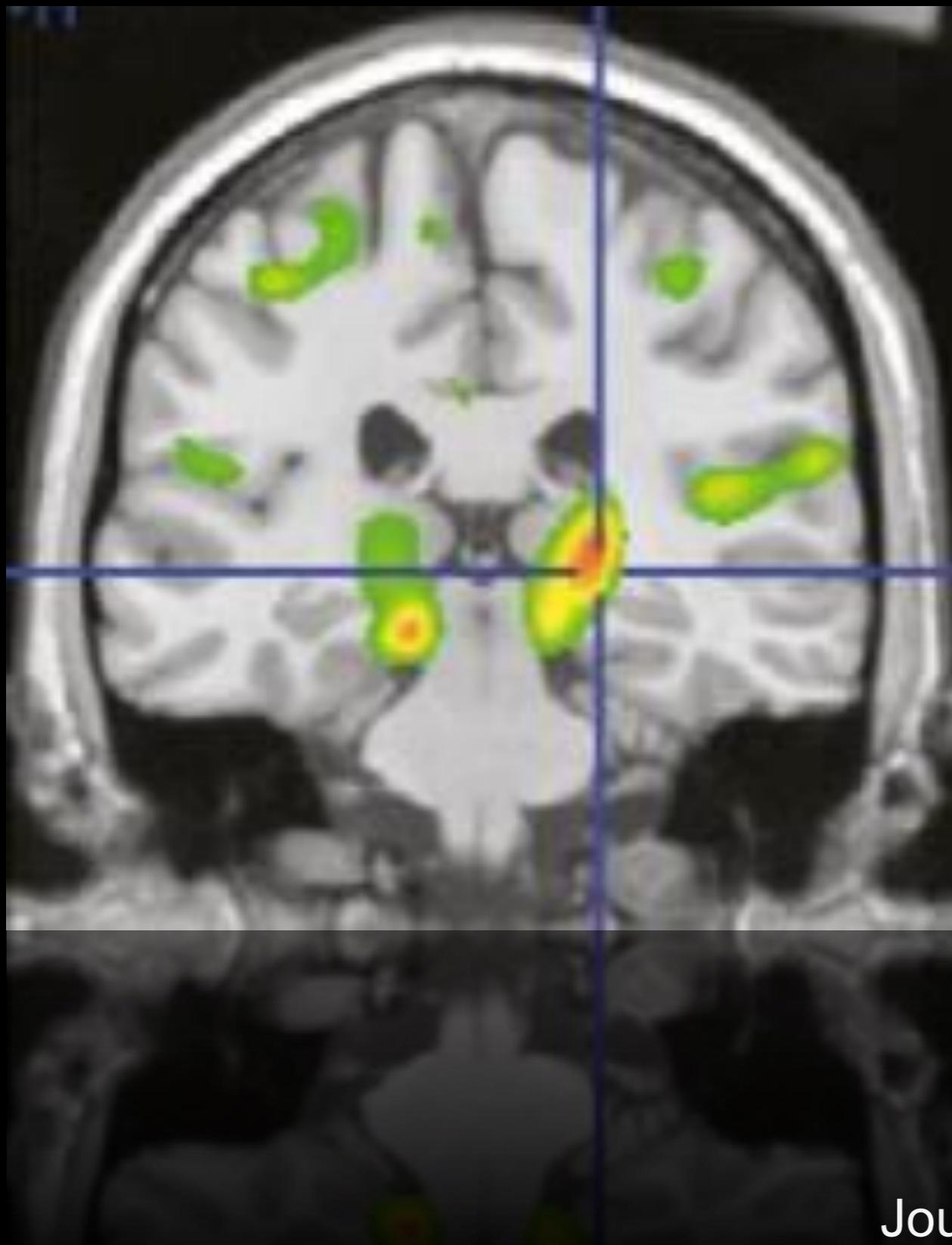
- 876 subjects, mean age 78.3 years
- 57.5% female, 42.5% male
- Weekly energy output (kcal) using the standardized Minnesota Leisure-Time Activities questionnaire
- Volumetric MR brain imaging

# Longitudinal Relationships between Caloric Expenditure and Gray Matter in the Cardiovascular Health Study

Higher energy output, from a variety of physical activity types, was associated with larger GM volumes in frontal, temporal, and parietal lobes, as well as hippocampus, thalamus, and basal ganglia.

Main effect of increasing caloric expenditure on gray matter structure. Red and yellow colors reflect larger gray matter volumes in the frontal, temporal, and parietal lobes





Main effect of increasing caloric expenditure on gray matter structure. Hotter colors denote a stronger effect and the cross hairs highlight the main effect of physical activity in the right hippocampus.

# Longitudinal Relationships between Caloric Expenditure and Gray Matter in the Cardiovascular Health Study

- The effects of physical activity did not vary as a function of race or education or gender.

# Longitudinal Relationships between Caloric Expenditure and Gray Matter in the Cardiovascular Health Study

- Studies such as this one suggest that simply caloric expenditure, regardless of type or duration of exercise, may alone moderate neurodegeneration and even increase GM volume in structures of the brain central to cognitive function.

- Individuals experiencing this brain benefit from increasing their physical activity experienced a 50% reduction in their risk of Alzheimer's dementia.
- Approximately 13% of AD cases worldwide may be attributable to sedentary behavior.
- A 25% reduction in sedentary behavior could potentially prevent more than 1 million AD cases globally.

# Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis

**Objective:** To determine if physical exercise is effective in improving cognitive function in adults over 50 years of age.

# Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis

**Design:** Meta-analysis of 39 studies.

# Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis

**Results:** Physical exercise improved cognitive function (0.29; 95% CI 0.17 to 0.41; p<0.01).

# Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis

**Results:** Interventions of aerobic exercise, resistance training, multicomponent training and tai chi, all had significant point estimates.

# Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis

**Results:** When exercise prescription was examined, a duration of 45–60 min per session and at least moderate intensity, were associated with benefits to cognition.

# Modulatory effect of coffee fruit extract on plasma levels of brain-derived neurotrophic factor in healthy subjects

- 25 subjects randomized
- no treatment, placebo (silica dioxide), grape seed extract, green coffee bean extract or whole coffee fruit extract (100 mg)
- BDNF at time 0, then every 30 minutes for 120 minutes

# BDNF (% change over initial) at 120 min



# BDNF

- Neurogenesis, neuronal differentiation, synaptogenesis
- Modulates appetite
- Enhances telomerase activity
- Involved in development, maintenance and function of the CNS
- Enhances long-term potentiation in the hippocampus
- Blocks injury related apoptosis
- Lower in Alzheimer's, Parkinson's, depression, obsessive-compulsive disorder

# Neurogenesis - Inflammation

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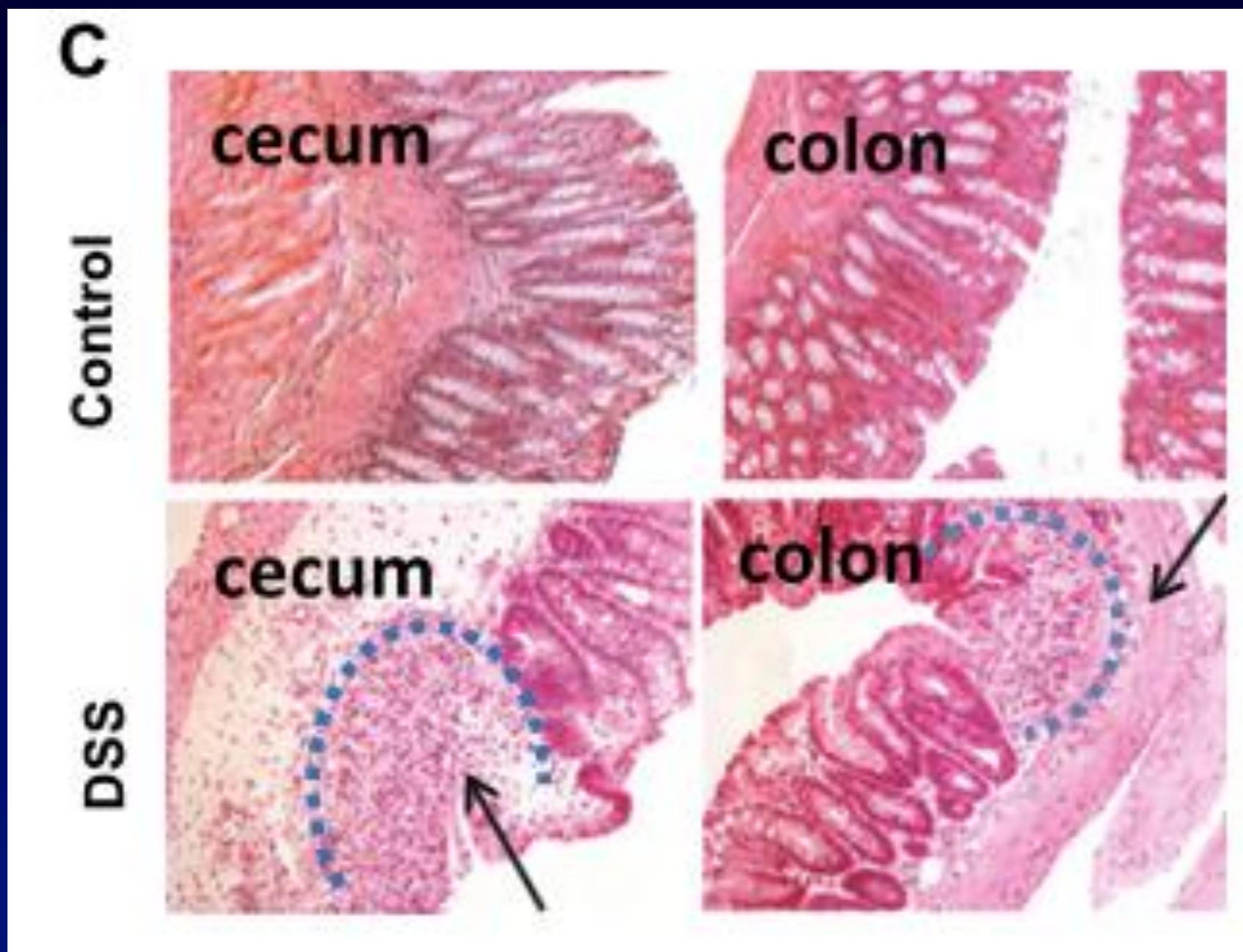
# Chronic intestinal inflammation alters hippocampal neurogenesis

Adult neurogenesis in the hippocampus is involved in learning, memory, and mood control. Decreased hippocampal neurogenesis elicits significant behavioral changes, including cognitive impairment and depression.

# Chronic intestinal inflammation alters hippocampal neurogenesis

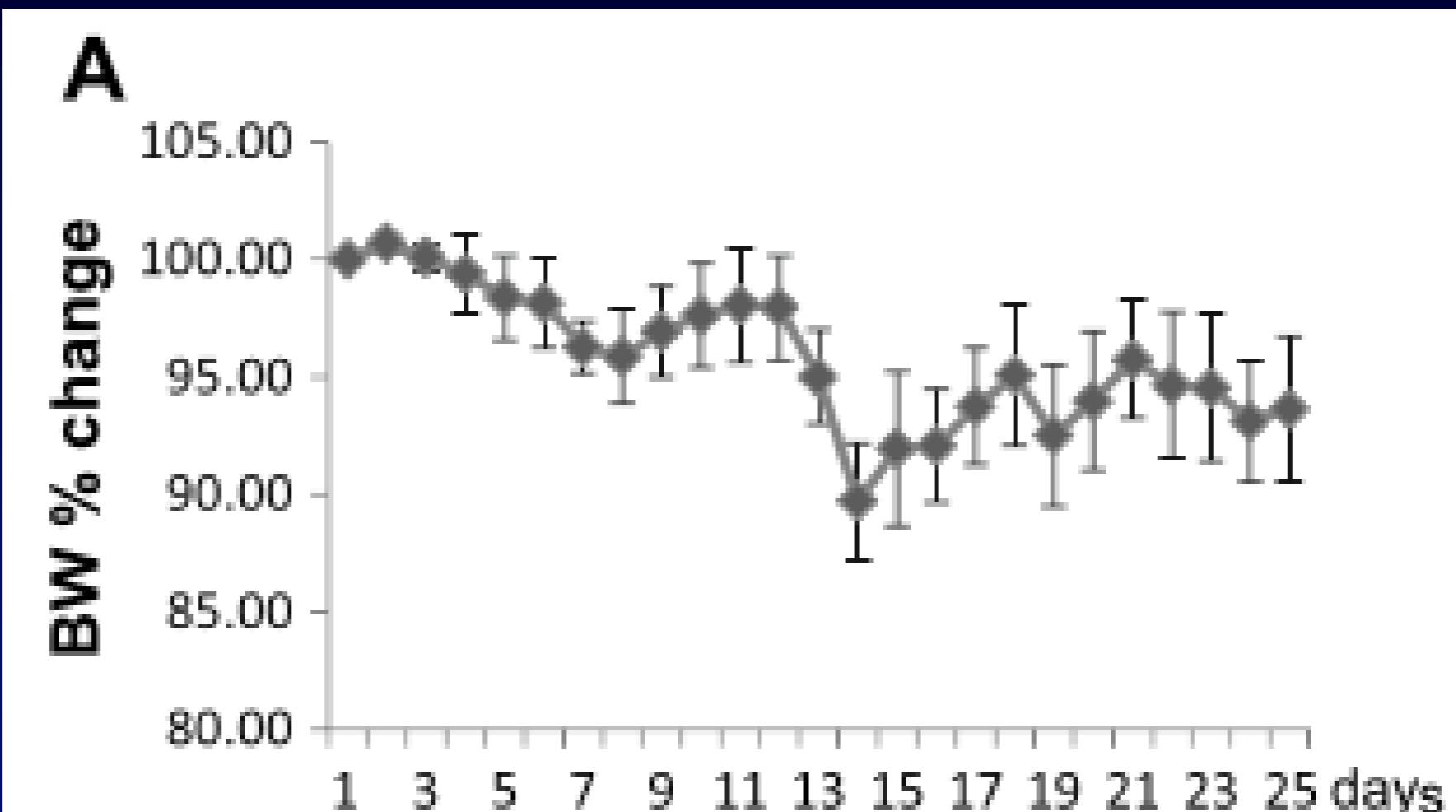
Dextran sodium sulfate (DSS) added to drinking water. Animals sacrificed day 7 and 29 (acute and chronic phases of inflammation)

# Day 29 after DSS administration

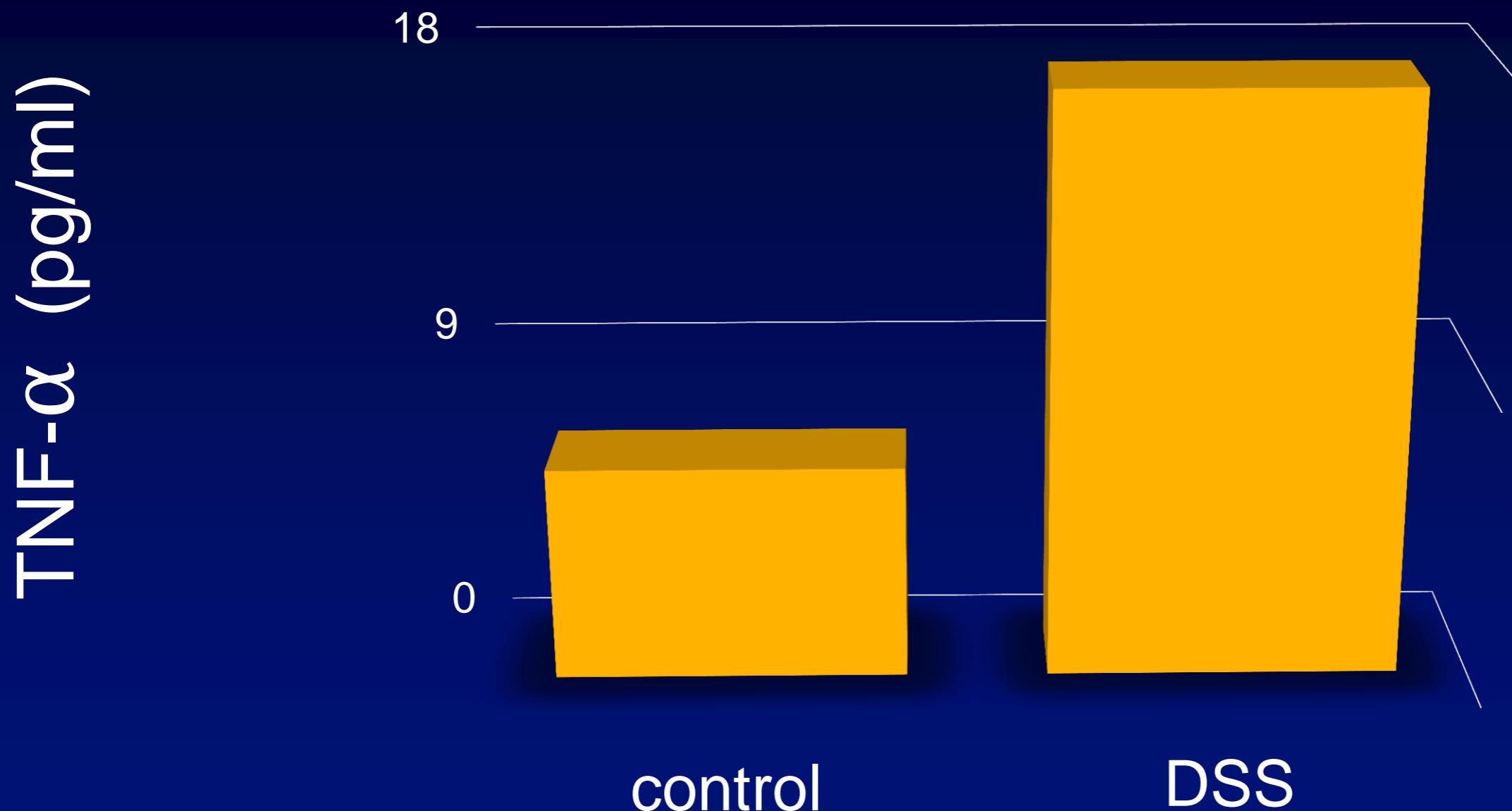


DSS

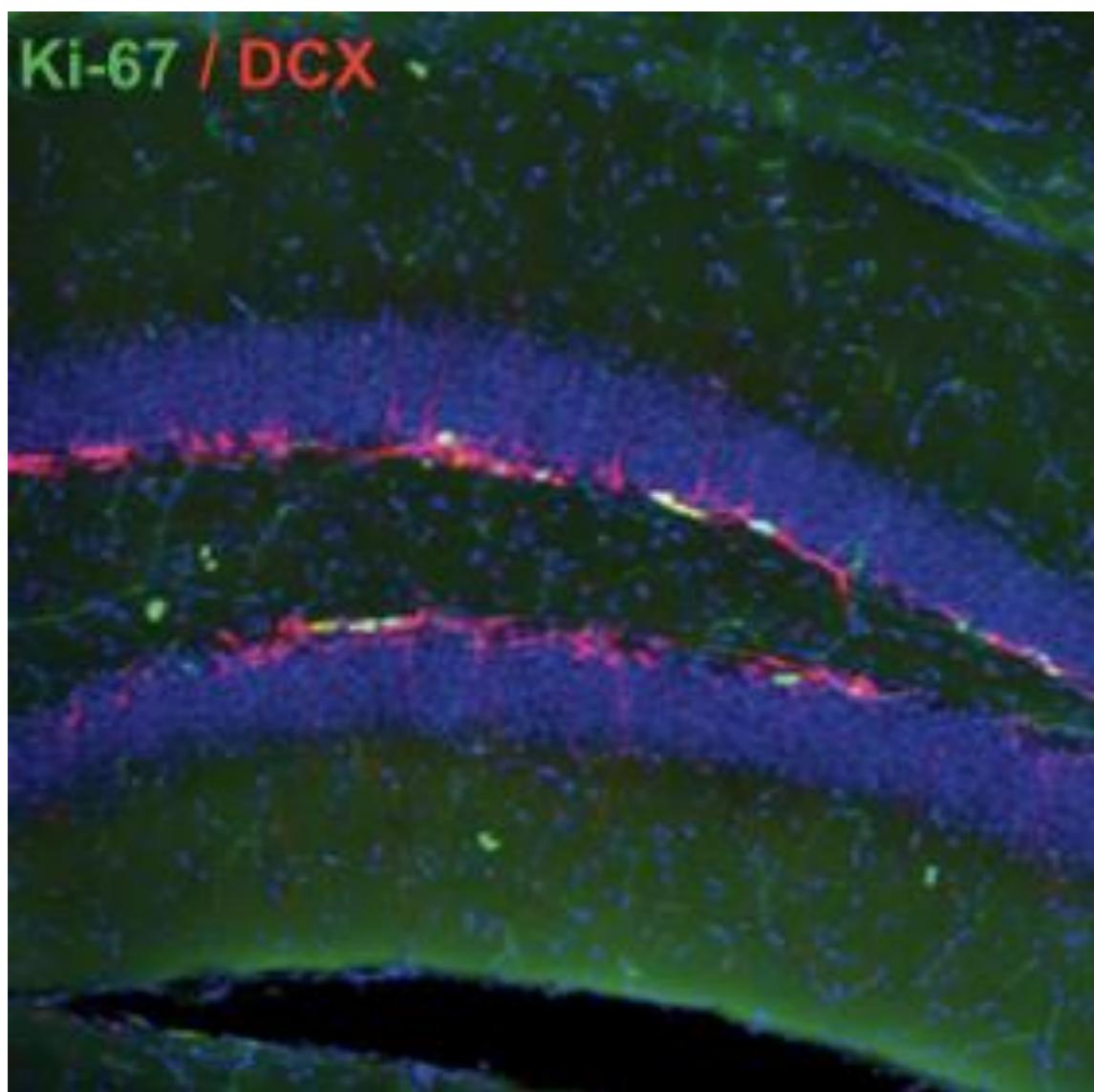
# Day 29 after DSS administration



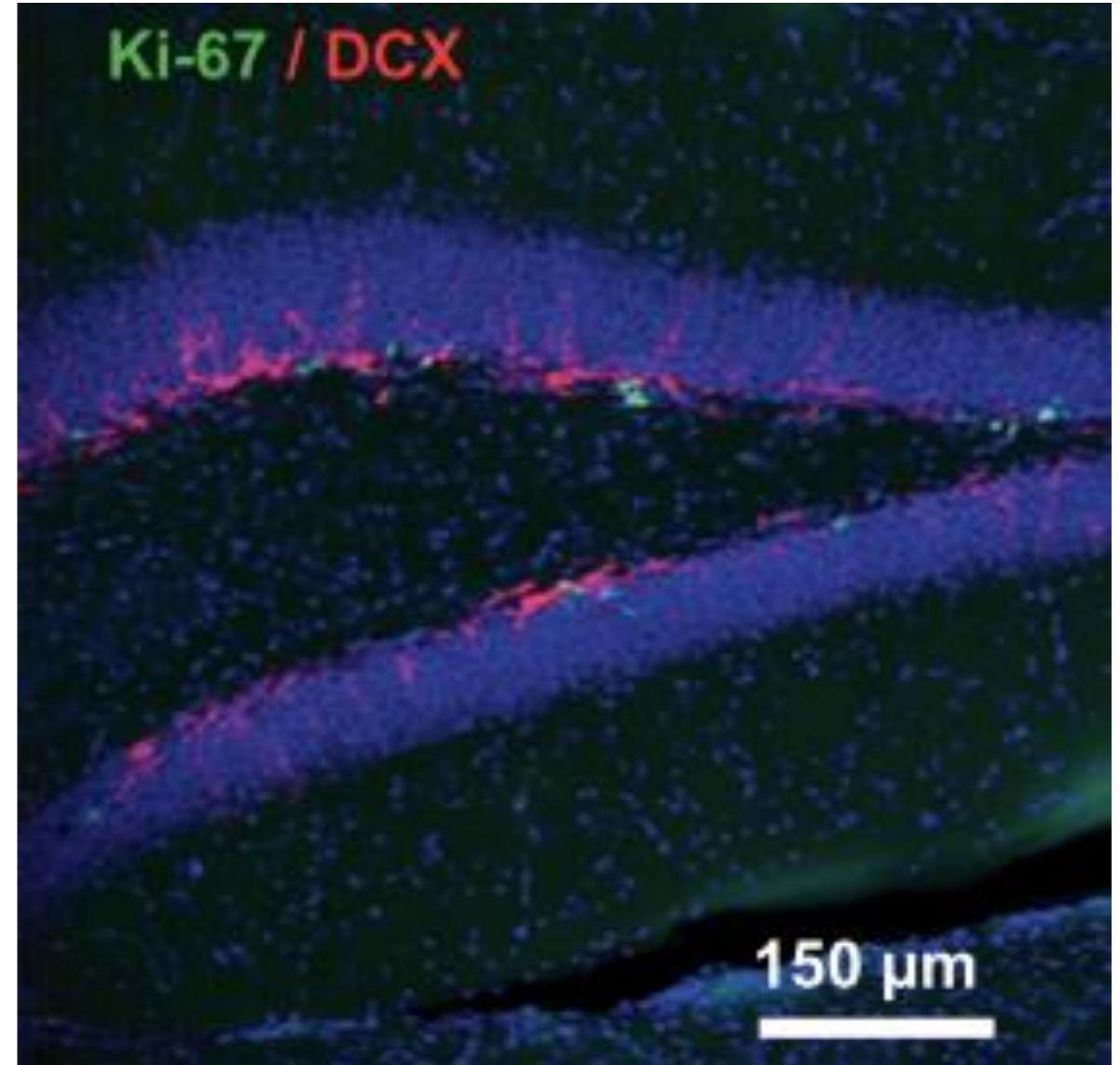
# Day 29 after DSS administration



# Day 29 after DSS administration

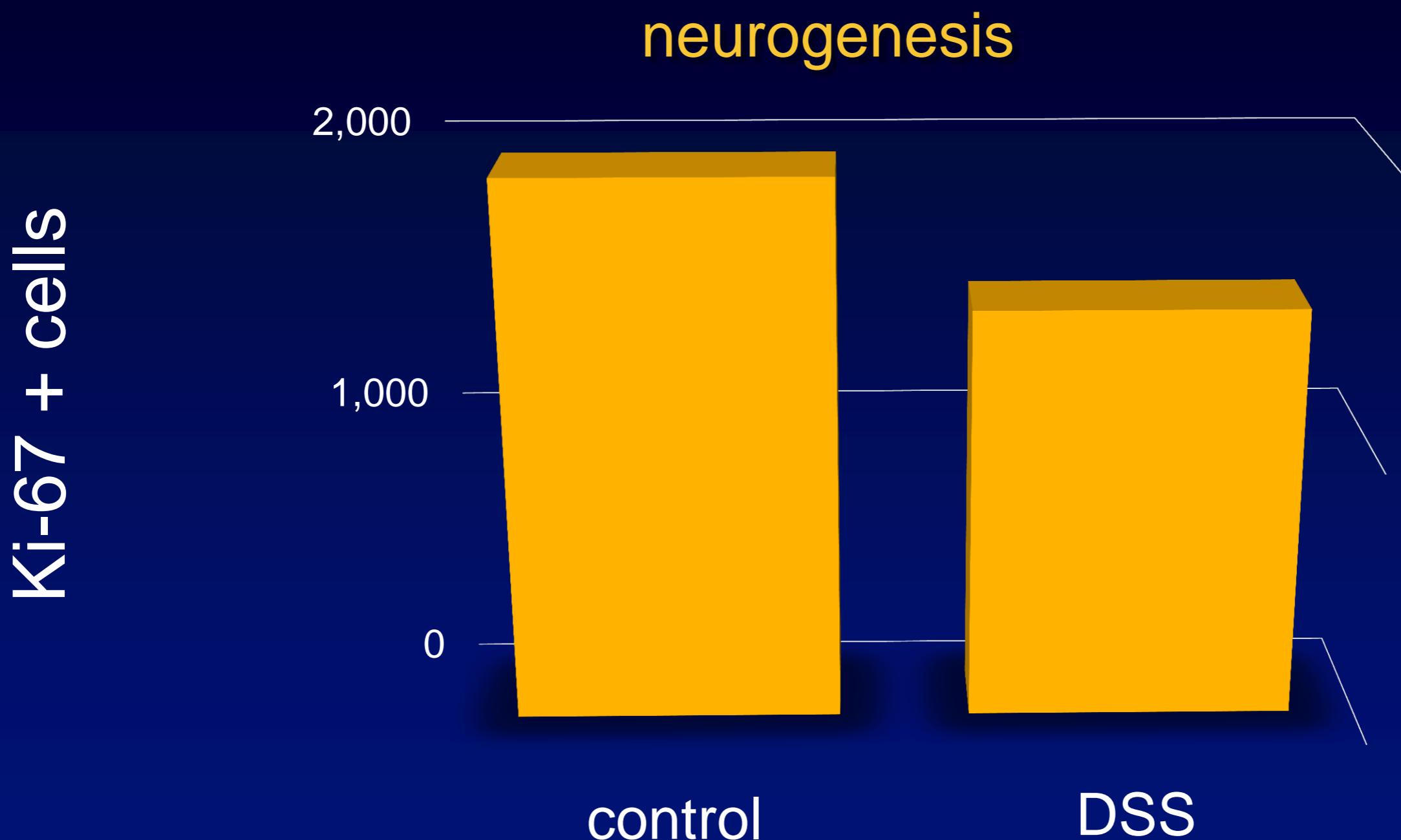


control



DSS

Day 29 after DSS administration



# Chronic intestinal inflammation alters hippocampal neurogenesis

Chronic intestinal inflammation suppresses hippocampal neurogenesis. Increased levels of proinflammatory cytokines have detrimental effects on proliferation of progenitors of neuronal lineage. Deficient hippocampal neurogenesis may underlie increased rate of mood disorder and cognitive impairment observed in IBD patients.

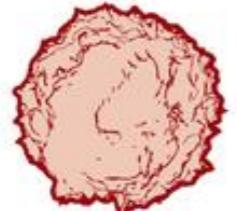
# THE POLLUTED BRAIN

- Smaller particles,  $< 2.5\mu\text{m}$  (PM 2.5) are most toxic and least regulated
- Associated with higher oxidative stress (peroxides) and pro-inflammatory cytokines
- Exposure is associated with asthma, lung cancer, coronary artery disease

## Modes of attack

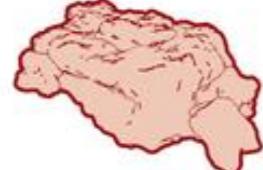
Pollutant particles might make their way to the brain and damage it directly, or they might attack it from a distance, by triggering the release of inflammatory molecules.

Industrial waste

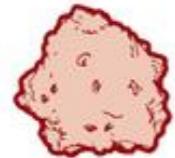


1  $\mu\text{m}$

Ash

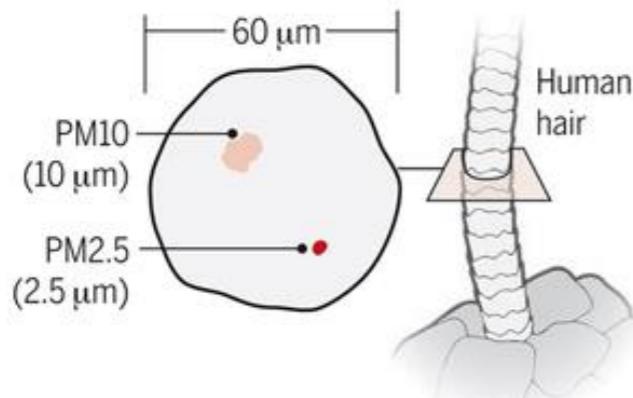


Fossil fuels



### Beyond fine

Pollutant particles are classified and regulated by size, although "ultrafine" pollutants of about  $0.2 \mu\text{m}$  are unregulated. The smaller the particle, the more damage it may do the brain.



Olfactory bulb

1

### Olfactory bulb transmission

Particles may enter the nose and travel through the olfactory bulb into the brain, directly seeding plaques and causing other problems.



### Adverse brain effects

2



### Nasal epithelial transmission

Particles may affect the lining of the nasal epithelium, producing inflammation that damages the brain.

3

### Mechanical inhalation

Particles that reach the lungs may inflame them, releasing brain-damaging cytokines.

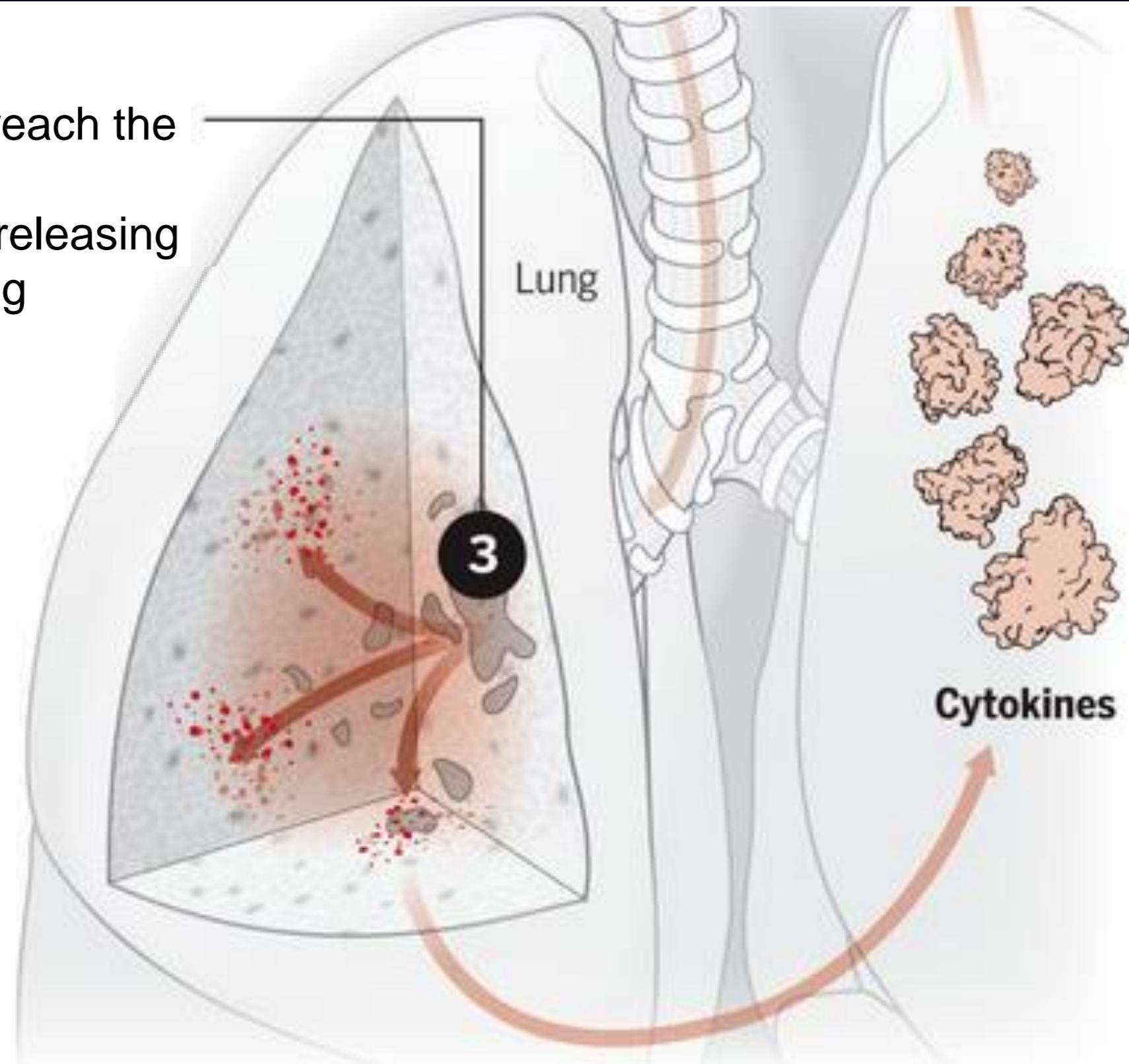
Lung

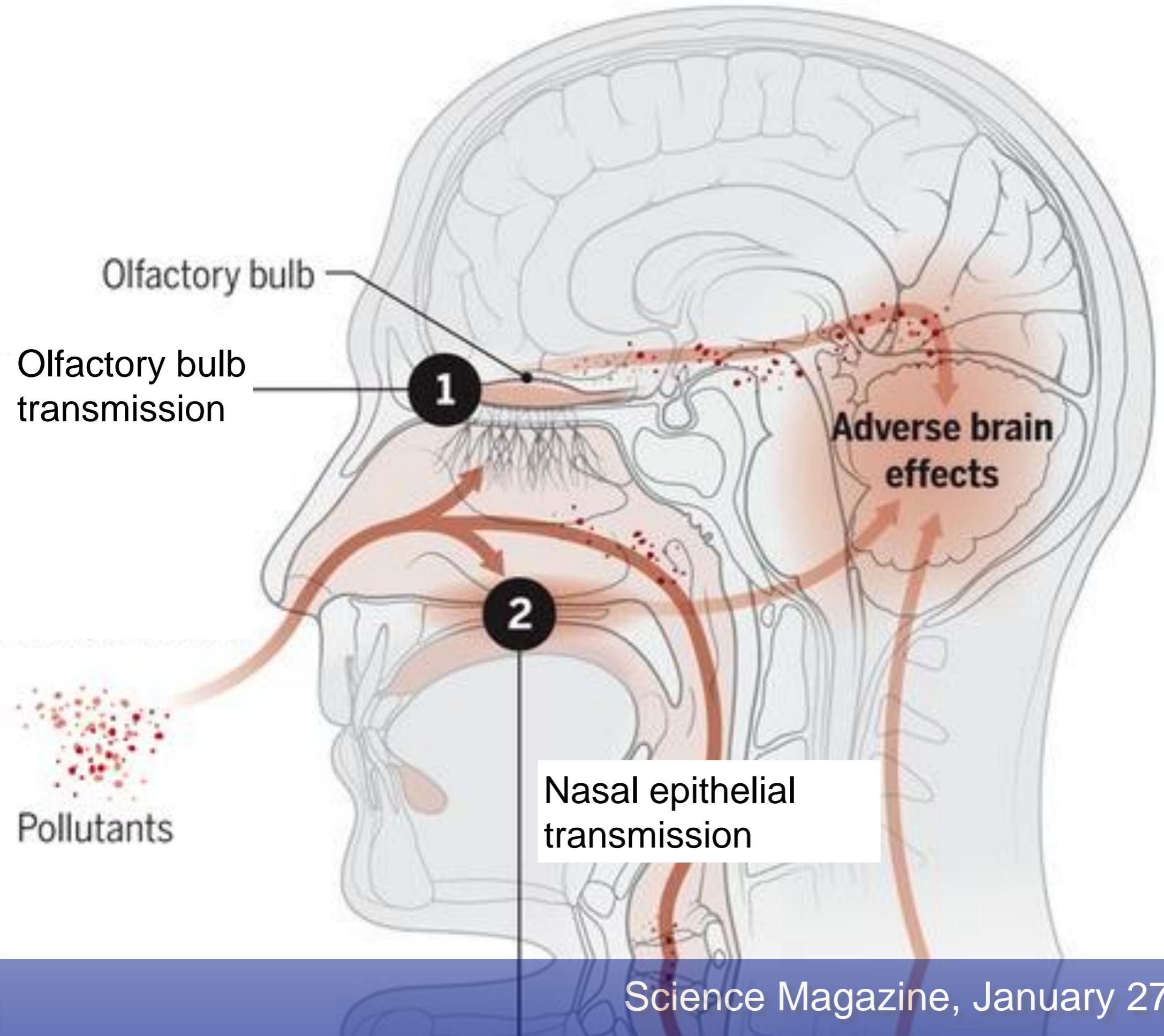


Cytokines

## Inhalation

Particles that reach the lungs produce inflammation, releasing brain-damaging **cytokines**.





# THE POLLUTED BRAIN

- *Lancet* report: risk of dementia increased 10-fold comparing those living within 50 meters of a major roadway compared to those living at least 150 meters away (6.6 million subjects, Ontario, Canada)

# THE POLLUTED BRAIN

- Harvard researchers using Framingham data shows higher PM 2.5 exposure creates with smaller brain volume

Prebiotic feeding elevates central brain derived neurotrophic factor, N methyl-D-aspartate receptor subunits and D-serine

Prebiotic feeding elevates central brain derived neurotrophic factor, N methyl-D-aspartate receptor subunits and D-serine

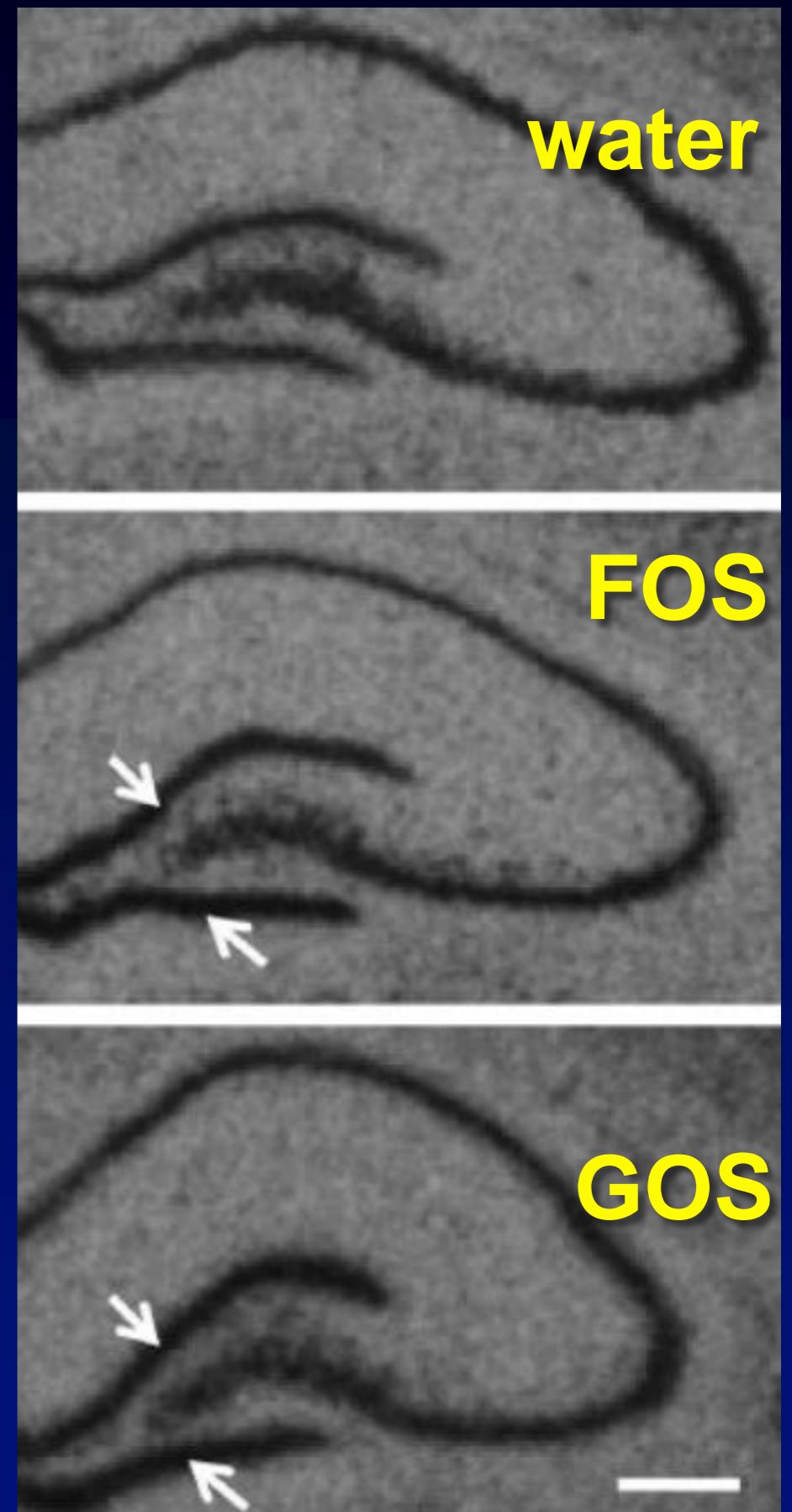
Rats gavaged for 5 weeks with:

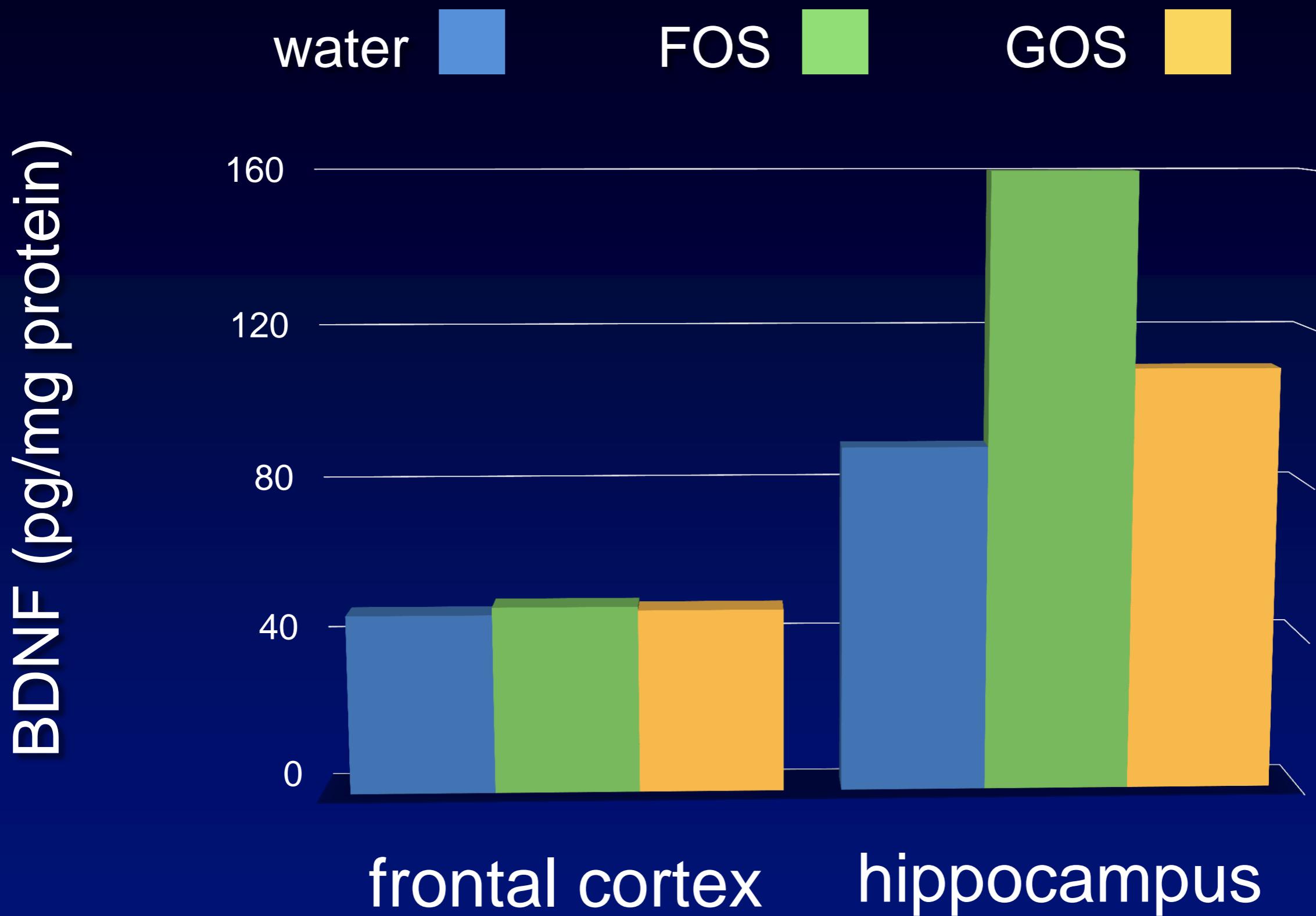
- Fructo-oligosaccarides (FOS)
- Galacto-oligosaccarides (GOS)
- Water

Prebiotic feeding elevates central brain derived neurotrophic factor, N methyl-D-aspartate receptor subunits and D-serine

- Measurement of BDNF
- Staining for BDNF in hippocampus

Staining of BDNF expression  
in dentate gyrus (arrows indicate  
increased expression)







# Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Alzheimer's Disease: A Randomized, Double-Blind and Controlled Trial

Elmira Akbari<sup>1</sup>, Zatollah Asemi<sup>2\*</sup>, Reza Daneshvar Kakhaki<sup>3</sup>, Fereshteh Bahmani<sup>1</sup>, Ebrahim Kouchaki<sup>3</sup>, Omid Reza Tamaji<sup>1</sup>, Gholam Ali Hamidi<sup>1</sup> and Mahmoud Salami<sup>1\*</sup>

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doi: 10.3389/fnagi.2016.00256

Alzheimer's disease (AD) is associated with severe cognitive impairments as well as some metabolic defects. Scant studies in animal models indicate a link between probiotics and cognitive function. This randomized, double-blind, and controlled clinical trial was conducted among 60 AD patients to assess the effects of probiotic supplementation on cognitive function and metabolic status. The patients were randomly divided into two groups ( $n = 30$  in each group) treating with either milk (control group) or a mixture of probiotics (probiotic group). The probiotic supplemented group took 200 ml/day probiotic milk containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus fermentum* ( $2 \times 10^9$  CFU/g for each) for 12 weeks. Mini-mental state examination (MMSE) score was recorded in all subjects before and after the treatment. Pre- and post-treatment fasting blood samples were obtained to determine the related markers. After 12 weeks intervention, compared with the control group ( $-5.03\% \pm 3.00$ ), the probiotic treated ( $+27.90\% \pm 8.07$ ) patients showed a significant improvement in the MMSE score ( $P < 0.001$ ). In addition, changes in plasma malondialdehyde ( $-22.01\% \pm 4.84$  vs.  $+2.67\% \pm 3.86 \mu\text{mol/L}$ ,  $P < 0.001$ ), serum high-sensitivity C-reactive protein ( $-17.61\% \pm 3.70$  vs.  $+45.26\% \pm 3.50 \mu\text{g/mL}$ ,  $P < 0.001$ ), homeostasis model of assessment-estimated insulin resistance ( $+28.84\% \pm 13.34$  vs.  $+76.95\% \pm 24.60$ ,  $P = 0.002$ ), Beta cell function ( $+3.45\% \pm 10.91$  vs.  $+75.62\% \pm 23.18$ ,  $P = 0.001$ ), serum triglycerides ( $-20.29\% \pm 4.49$  vs.  $-0.16\% \pm 5.24 \text{ mg/dL}$ ,  $P = 0.003$ ), and quantitative insulin sensitivity check index ( $-1.83 \pm 1.26$  vs.  $-4.66 \pm 1.70$ ,  $P = 0.006$ ) in the probiotic group were significantly varied compared to the control group. We found that the probiotic treatment had no considerable effect on other biomarkers of oxidative stress and inflammation, fasting plasma glucose, and other lipid profiles. Overall, the current study demonstrated that probiotic consumption for 12 weeks positively affects cognitive function and some metabolic statuses in the AD patients. Clinical Trial Registration: <http://www.irct.ir/>, IRCT201511305623N60.

**Keywords:** Alzheimer's disease, clinical trial, cognitive function, metabolic status, probiotic

# Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Alzheimer's Disease: A Randomized, Double-Blind and Controlled Trial

- 60 adults, age 60-95 years with confirmed AD
- Placebo or probiotic
- 12 week study
- Pre and post MMSE

# Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Alzheimer's Disease: A Randomized, Double-Blind and Controlled Trial

- Intervention: *Lactobacillus acidophilus*,  
*Lactobacillus casei*, *Bifidobacterium bifidum*, and  
*Lactobacillus fermentum*

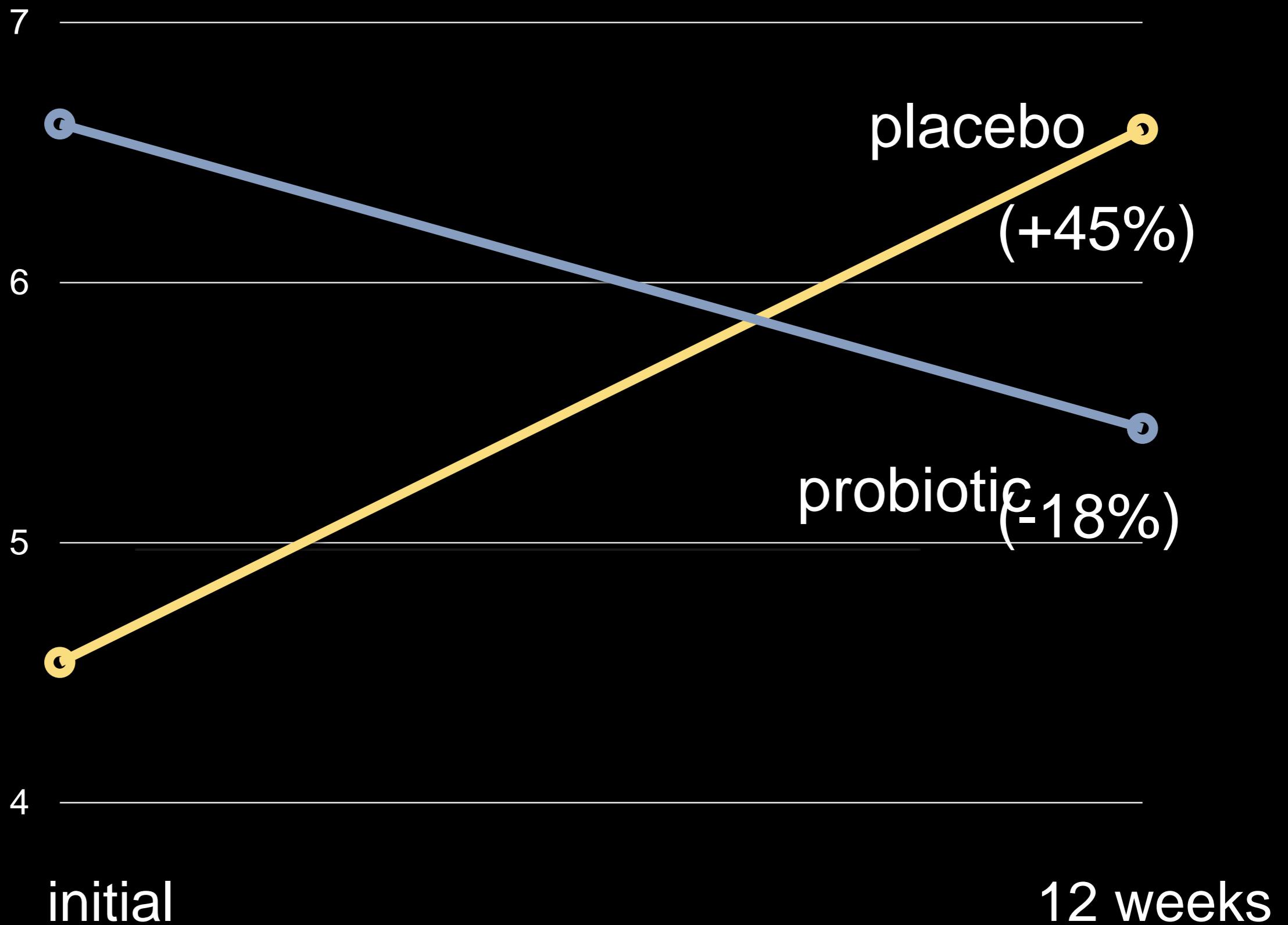
# Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Alzheimer's Disease: A Randomized, Double-Blind and Controlled Trial

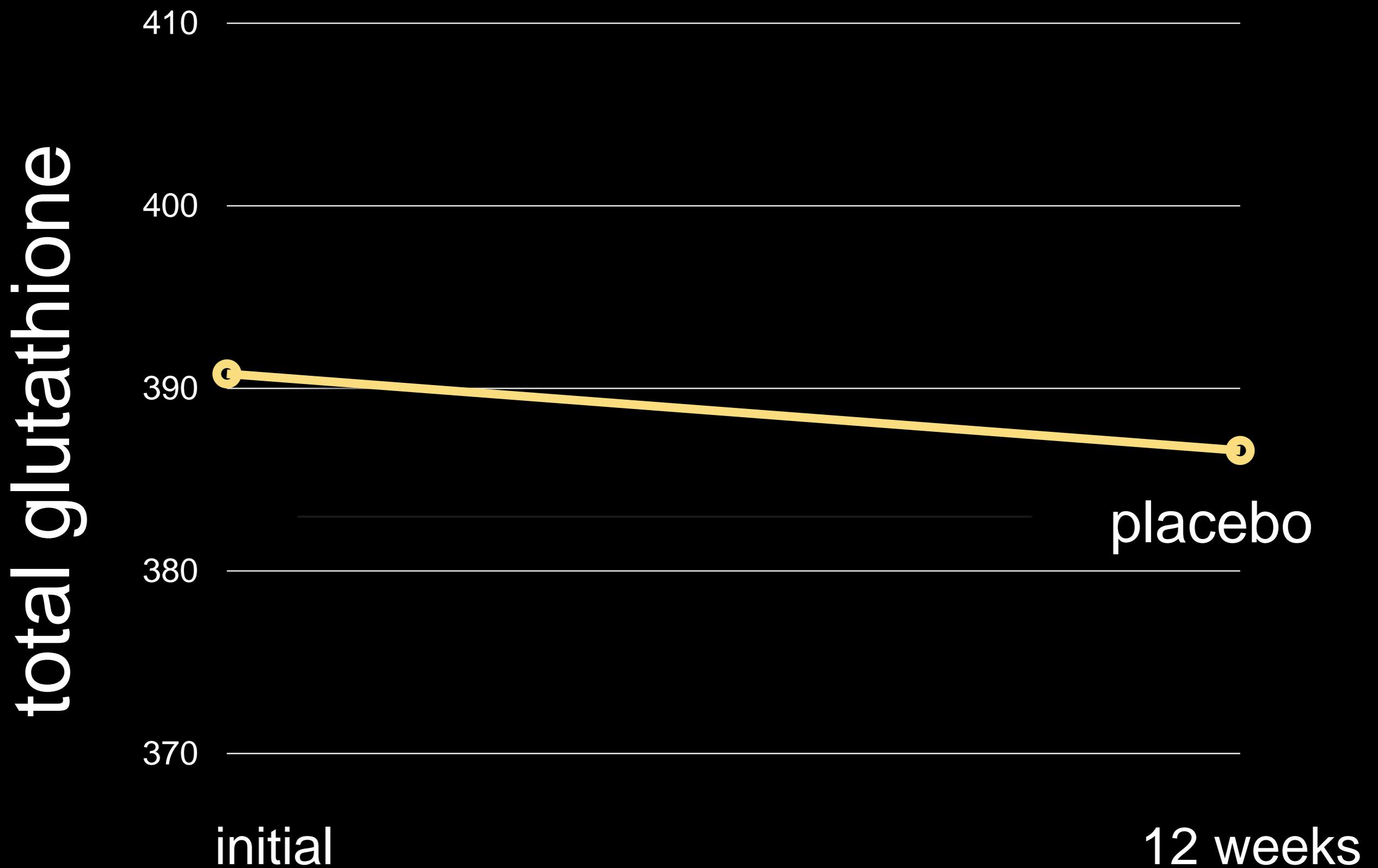
- hs-CRP
- total glutathione
- beta cell function

hs-CRP

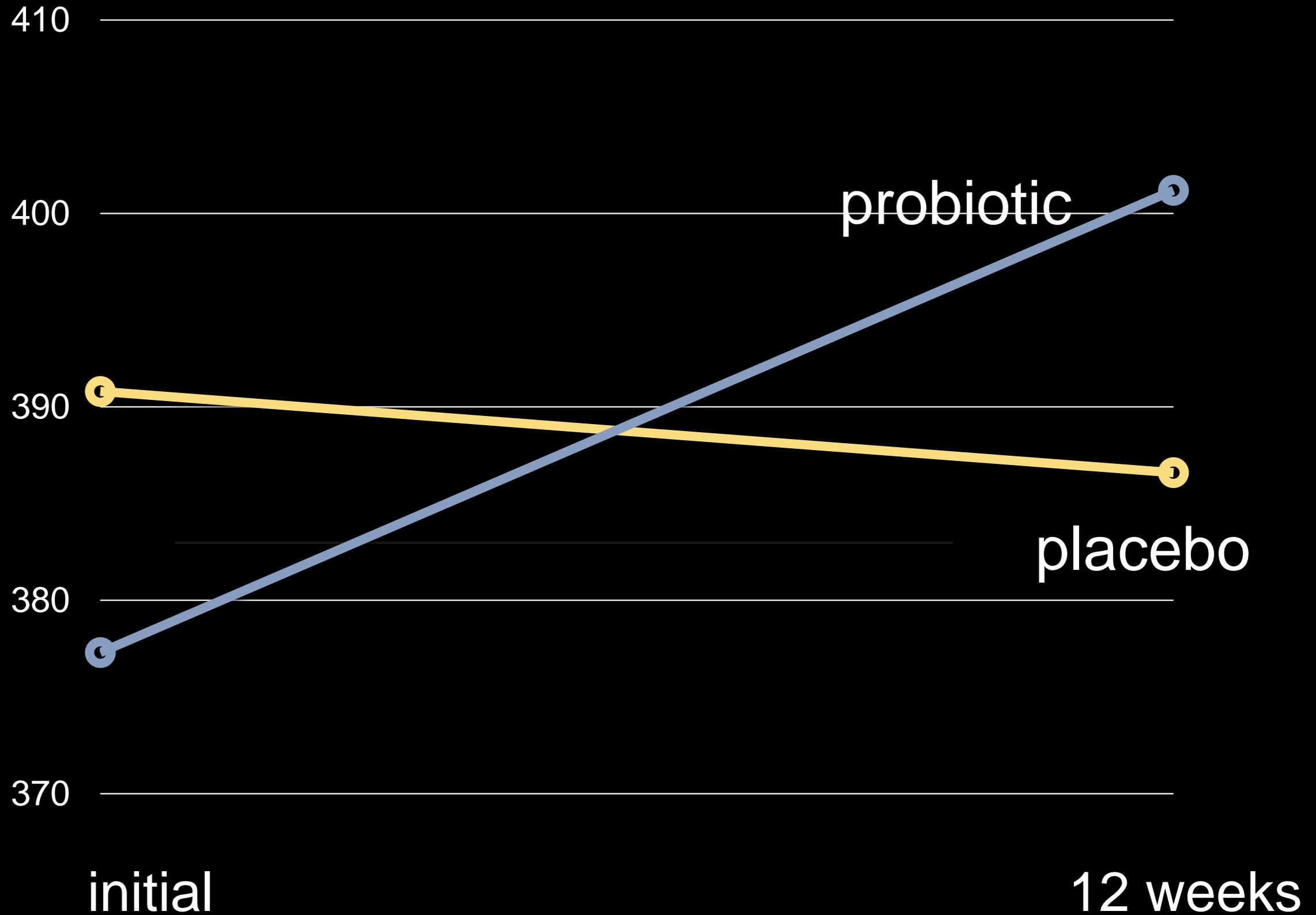


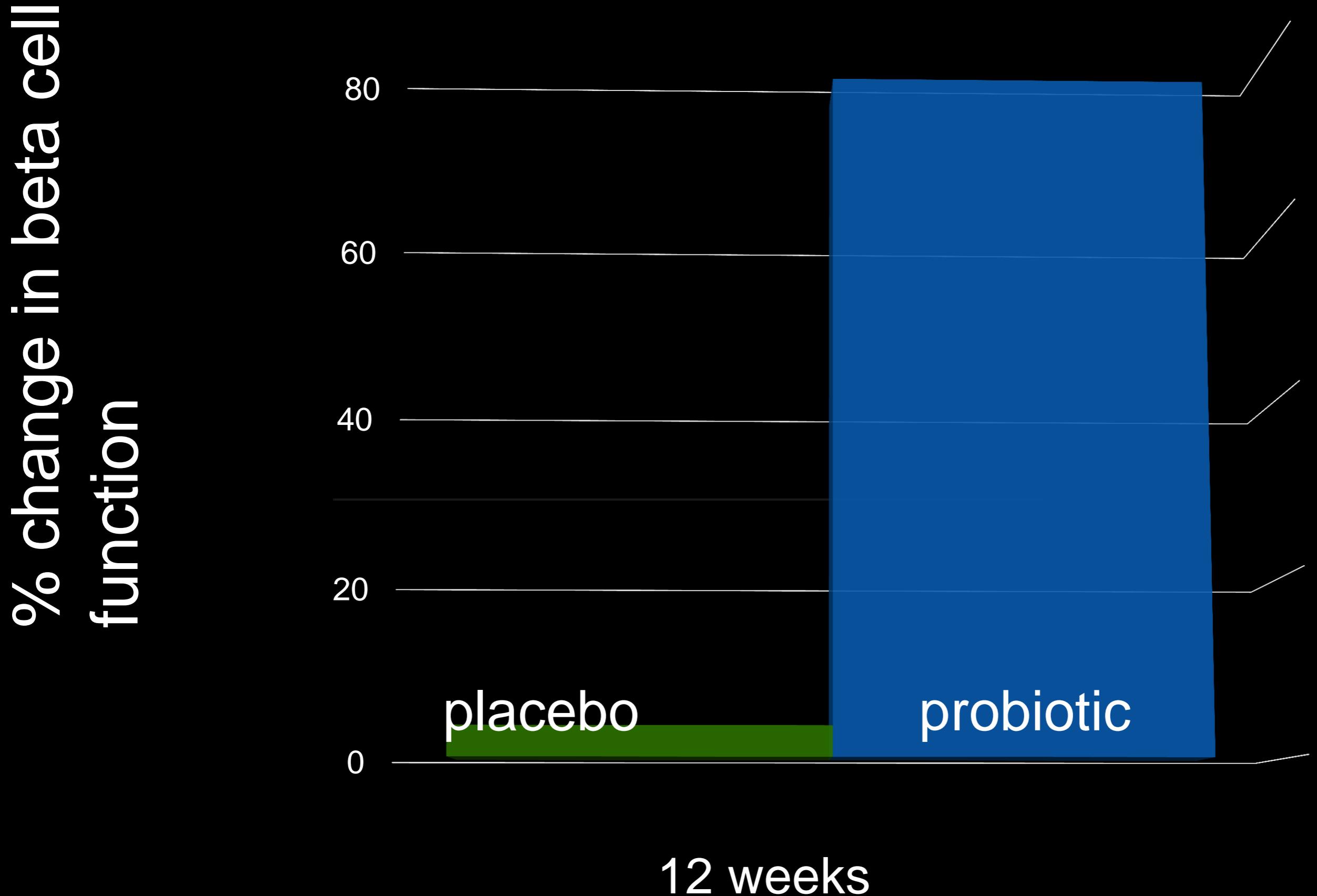
hs-CRP



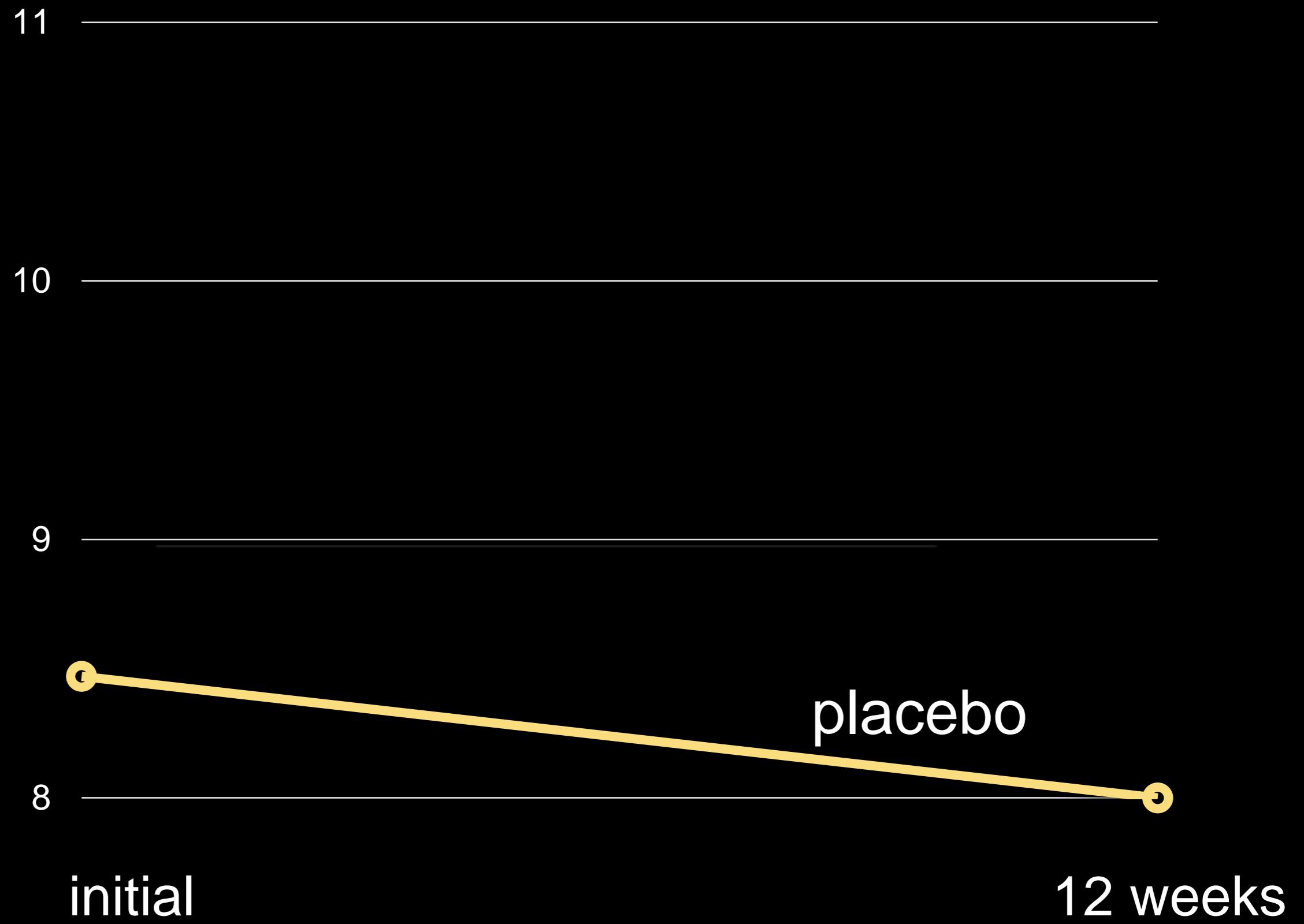


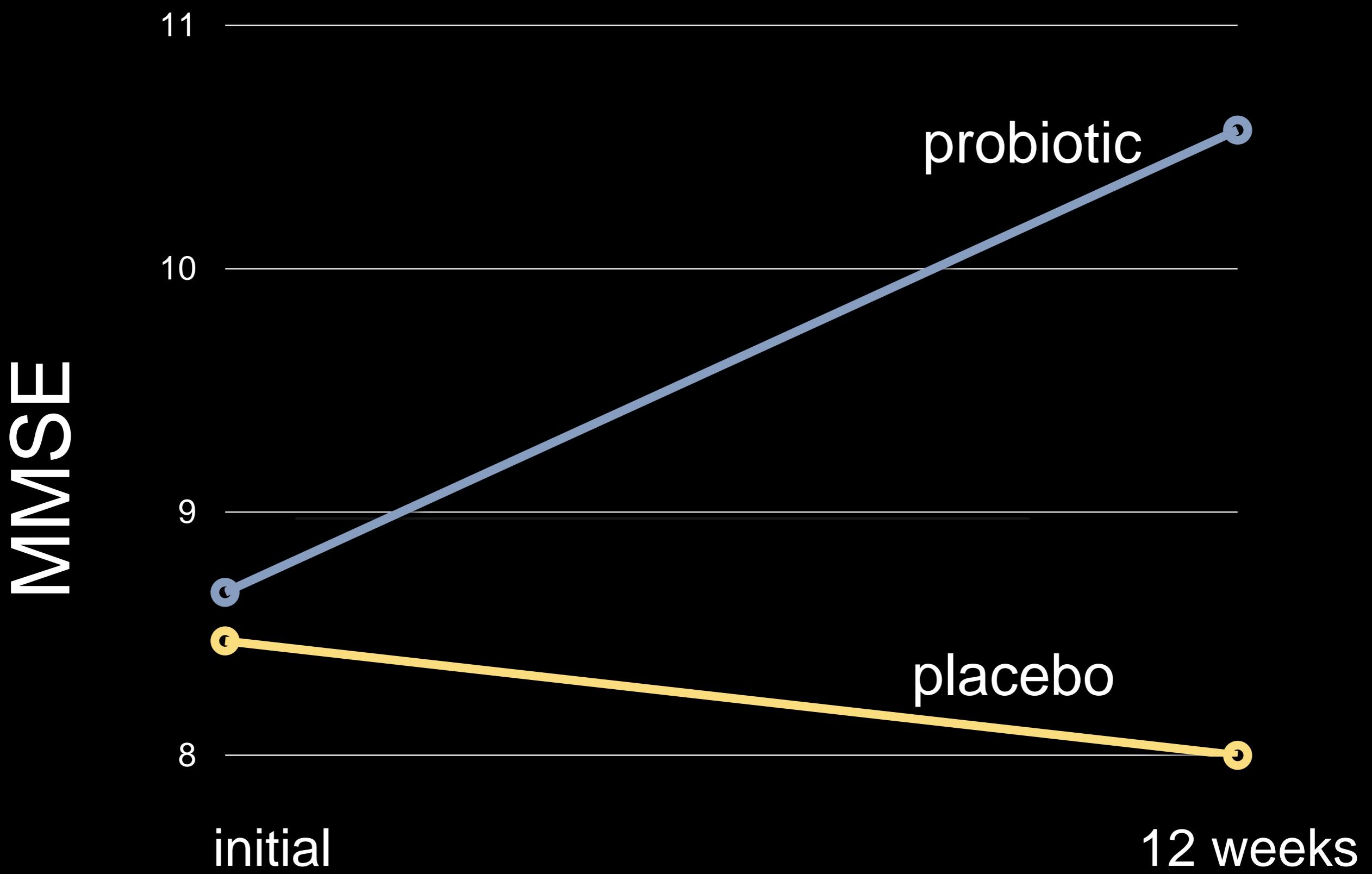
total glutathione





MMSE





# Neurogenesis - Inflammation

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# Inflammation



- Alzheimer's disease
- Parkinson's disease
- Autism
- Multiple sclerosis
- Stroke
- Depression
- ADHD

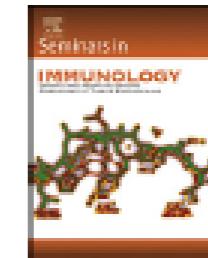
# Commensal flora and the regulation of inflammatory and autoimmune responses and autoimmunity



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### Review

## Commensal flora and the regulation of inflammatory and autoimmune responses and autoimmunity

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### ARTICLE INFO

#### Keywords:

Gut bacteria

Commensals

Microbiota

Autoimmunity

Symbiosis factors

Dysbiosis

### ABSTRACT

The gut microbiota has recently been recognized for its role in immune regulation, and changes in gut microbiota may be the basis for an increased incidence of autoimmune diseases and asthma in developed countries. Beneficial microbes produce factors that are distributed systemically, and therefore can influence peripheral inflammatory responses. Such symbiosis factors are important for the control and resolution of inflammation and autoimmune diseases. Here we discuss immune regulation by recently identified symbiosis factors and how certain environmental factors favor their production and influence the composition of the gut microflora.

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Keywords:  
Symbiosis factors  
Gut microbiota  
Autoimmunity  
Microbiota  
Commensals  
Dysbiosis

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The symbiosis of the gut microbiota  
produces factors that are distributed systemically, and therefore can influence peripheral inflammatory responses. Such symbiosis factors are important for the control and resolution of inflammation and autoimmune diseases. Here we discuss immune regulation by recently identified symbiosis factors and how certain environmental factors favor their production and influence the composition of the gut microflora.

# Obesity and Gut's Dysbiosis Promote Neuroinflammation, Cognitive Impairment, and Vulnerability to Alzheimer's disease: New Directions and Therapeutic Implications

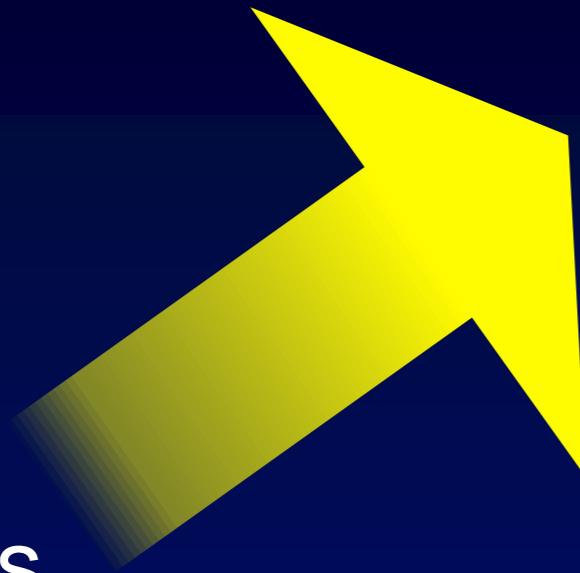
- Obesity is associated with inflammation
- Obesity is associated with hippocampal atrophy
- Obesity is associated with cognitive decline

# Obesity and Gut's Dysbiosis Promote Neuroinflammation, Cognitive Impairment, and Vulnerability to Alzheimer's disease: New Directions and Therapeutic Implications

- Obesity is associated with <sup>dysbiosis</sup> dysbiosis

“This may be enhanced by concomitant noxious factors such as consumption of NSAIDS.”

dysbiosis



gut inflammation

increased gut permeability

translocation of LPS

pro-inflammatory cytokines

neuro-inflammation



dysbiosis

gut inflammation

increased gut permeability

translocation of LPS

pro-inflammatory cytokines

neuro-inflammation

# Threaten microbial diversity

- Antibiotics
- Method of delivery
- Medications
- Water treatment
- Diet
- Stress
- GMO

leading to increased gut permeability

# Cardiorespiratory fitness as a predictor of intestinal microbiome

- Reduced diversity - autoimmune, metabolic, and inflammatory diseases

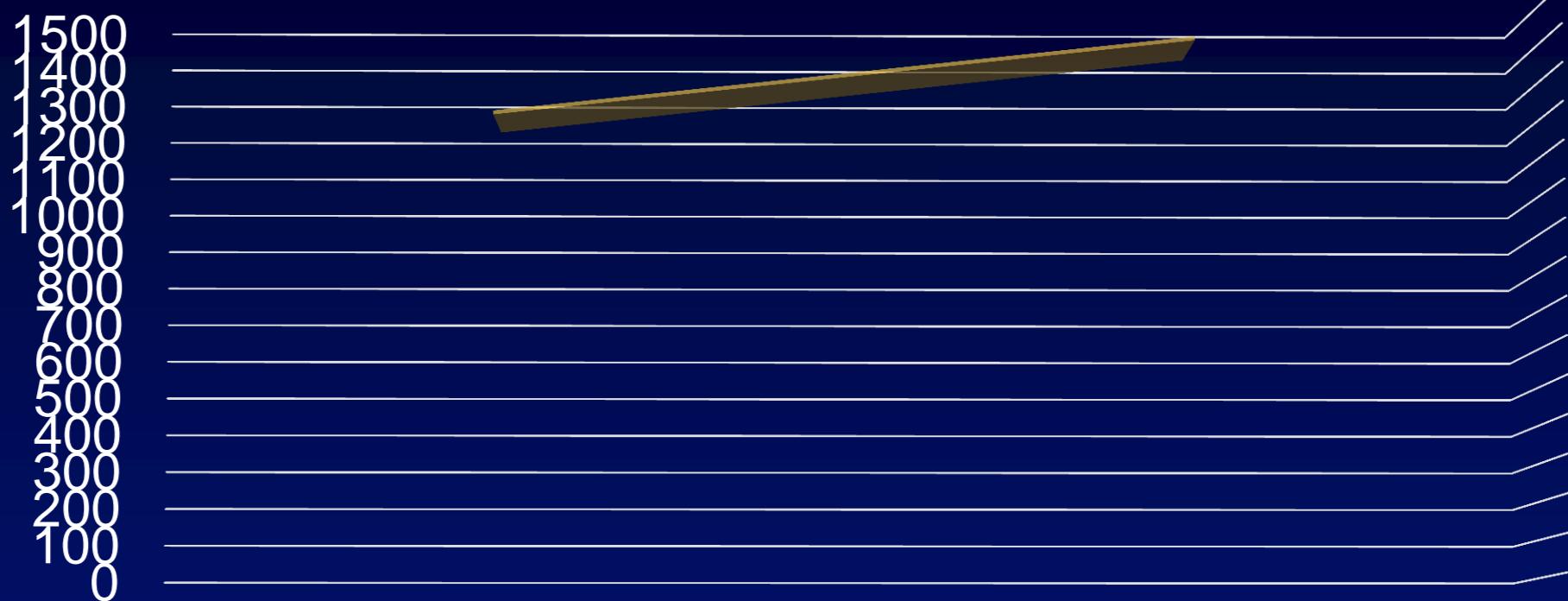
# Cardiorespiratory fitness as a predictor of intestinal microbi

- Diabetes (types 1 and 2), obesity, Alzheimer's, MS, autism, colorectal cancer, inflammatory bowel disease.

# Cardiorespiratory fitness as a predictor of intestinal microbiome

- Analysis of fecal microbiota of 39 healthy participants with similar age, BMI, and diets but with varying cardiorespiratory fitness levels.
- Correlated with peak oxygen uptake ( $\text{VO}_2$  peak), the gold standard measure of cardiorespiratory fitness.

species diversity



peak VO<sub>2</sub>

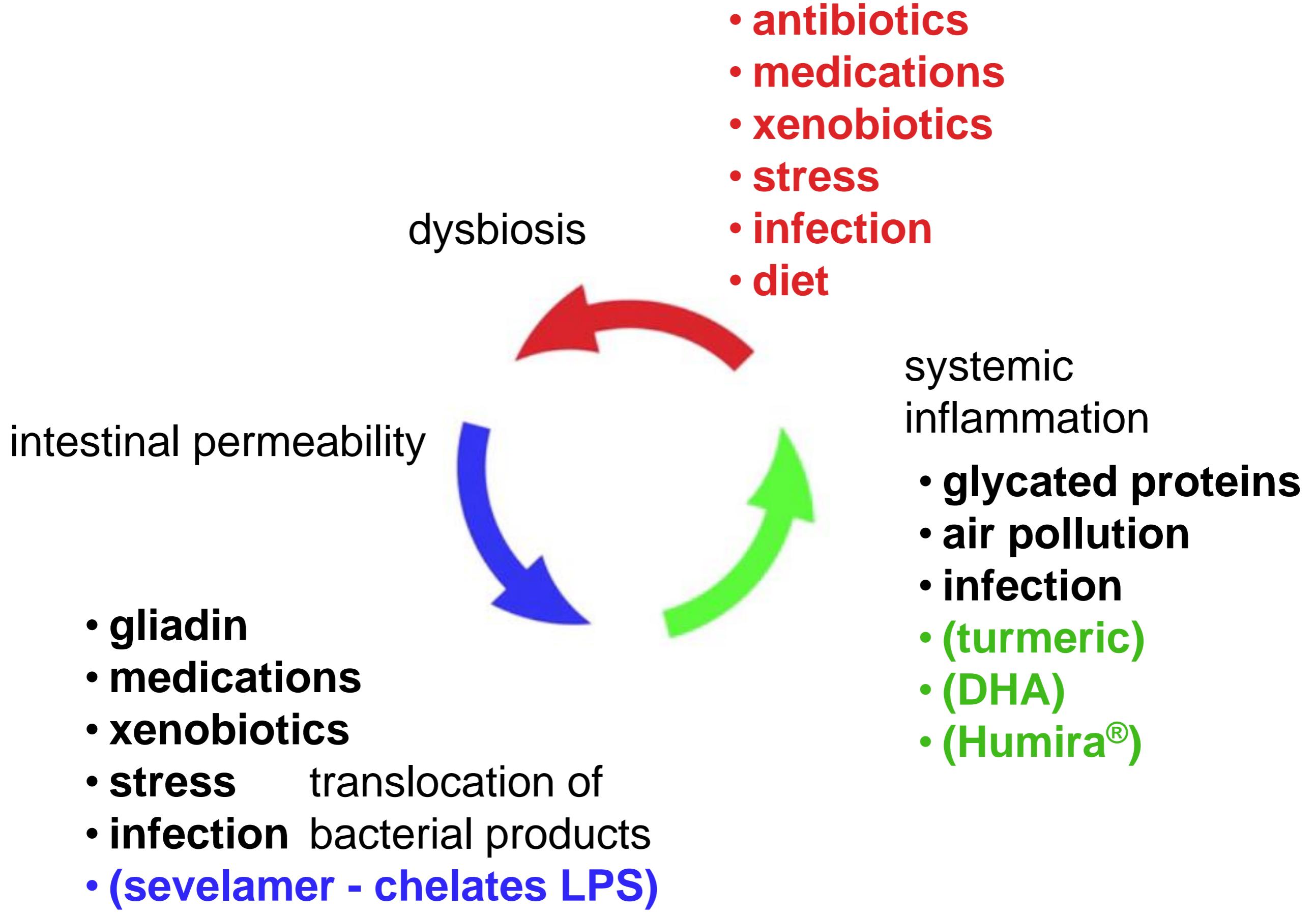
# Cardiorespiratory fitness as a predictor of intestinal microbiome

- Our regression model showed that ~20 % of variation in gut bacterial alpha diversity could be explained by VO<sub>2</sub> peak alone; in fact, VO<sub>2</sub> peak stood as the *only* variable that significantly contributed to increased alpha diversity. The primary findings from this study suggest that cardiorespiratory fitness is a good predictor of gut microbial diversity in healthy humans, outperforming several other variables including sex, age, BMI, and dietary components.

# Cardiorespiratory fitness as a predictor of intestinal microbiome

- The microbiome in high cardiorespiratory fitness individuals seems to favor a decreased LPS biosynthetic pathways. In addition, a strong positive correlation was observed between  $\text{VO}_2$  peak and fecal butyric acid, a SCFA associated with gut health.

Tissue destruction and senescence are the consequences of chronic systemic inflammation which occurs as a result of increased permeability in the colon and the escape of bacteria and their products.



# Potential Adverse Effects of Proton Pump Inhibitors in the Elderly

Specifically, increasing evidence demonstrates that PPI therapy may be associated with the development of:

- *Clostridium difficile* infections
- hip fractures
- community acquired pneumonia
- vitamin B12 deficiency
- allergic reactions

# Potential Adverse Effects of Proton Pump Inhibitors in the Elderly

The etiology of these side effects, particularly diarrhea, may be related to alterations in gut flora caused by acid suppression.

# IF YOU SUFFER FROM FREQUENT HEARTBURN, I'VE GOT SOMETHING FOR YOU TO TRY.

Prilosec OTC® treats frequent heartburn by blocking the acid that causes it, so you don't get heartburn in the first place.\*

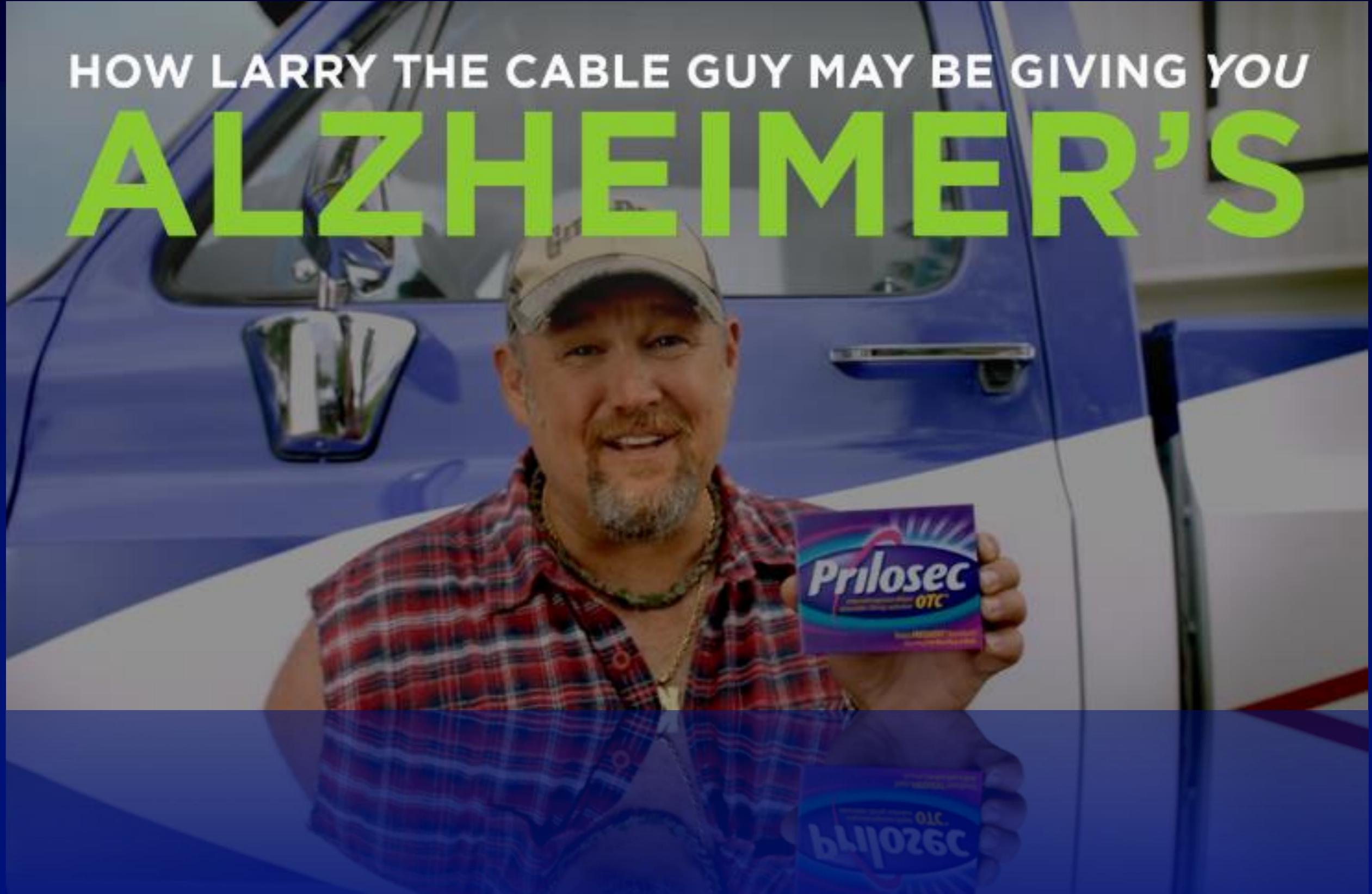
LARRY THE CABLE GUY  
ACTUAL USER

YOU ARE THE CABLE  
EAT JACKET

\*As sold in the United States, Prilosec OTC  
only as it is used to treat heartburn. It is not  
intended to treat acid reflux disease.



HOW LARRY THE CABLE GUY MAY BE GIVING YOU  
**ALZHEIMER'S**



Original Investigation

# Association of Proton Pump Inhibitors With Risk of Dementia A Pharmacoepidemiological Claims Data Analysis

Ulrich Gomber, PhD; Susanne von Holt, MD, PhD; Jürgen Thonke, MD; Michael M. Haenisch, MD; Britta Haenisch, PhD; Anne Fink, MSc; Gottlieb Dobhammer, PhD; Britta Haenisch, PhD

**IMPORTANCE:** Medications that influence the risk of dementia in the elderly can be relevant for dementia prevention. Proton pump inhibitors (PPIs) are widely used for the treatment of gastrointestinal diseases but have also been shown to be potentially involved in cognitive decline.

**OBJECTIVE:** To examine the association between the use of PPIs and the risk of incident dementia in the elderly.

**DESIGN, SETTING, AND PARTICIPANTS:** We conducted a prospective cohort study using observational data from 2004 to 2011, derived from the largest German statutory health insurer, Allgemeine Ortskrankenkassen (AOK). Data on inpatient and outpatient diagnoses (coded by the German modification of the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision) and drug prescriptions (categorized according to the Anatomical Therapeutic Chemical Classification System) were available on a quarterly basis. Data analysis was performed from August to November 2015.

**EXPOSURES:** Prescription of omeprazole, pantoprazole, lansoprazole, esomeprazole, or rabeprazole.

**MAIN OUTCOMES AND MEASURES:** The main outcome was a diagnosis of incident dementia coded by the German modification of the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision. The association between PPI use and dementia was analyzed using time-dependent Cox regression. The model was adjusted for potential confounding factors, including age, sex, comorbidities, and polypharmacy.

**RESULTS:** A total of 73 679 participants 75 years of age or older and free of dementia at baseline were analyzed. The patients receiving regular PPI medication ( $n = 2950$ ; mean [SD] age, 83.8 [5.4] years; 77.9% female) had a significantly increased risk of incident dementia compared with the patients not receiving PPI medication ( $n = 70 729$ ; mean [SD] age, 83.0 [5.6] years; 73.6% female) (hazard ratio, 1.44 [95% CI, 1.36-1.52];  $P < .001$ ).

**CONCLUSIONS AND RELEVANCE:** The avoidance of PPI medication may prevent the development of dementia. This finding is supported by recent pharmacoepidemiological analyses on primary data and is in line with mouse models in which the use of PPIs increased the levels of  $\beta$ -amyloid in the brains of mice. Randomized, prospective clinical trials are needed to examine this connection in more detail.

Editorial page 379

Supplemental content at  
[jamanetwork.com](http://jamanetwork.com)

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JAMA Neurol. 2016;73(4):410-416. doi:10.1001/jamaneurol.2015.4291  
Published online February 15, 2016.

# Association of Proton Pump Inhibitors With Risk of Dement

JAMA Neurology. February 15, 2016

# Association of Proton Pump Inhibitors With Risk of Dement

- 73,679 dementia free adults
- aged  $\geq$  75 years
- followed for 5.4 - 5.6 years

# Association of Proton Pump Inhibitors With Risk of Dement

Risk of dementia in regular users of PPI drugs was increased by 44%.

# Association of Proton Pump Inhibitors With Risk of Dement

“Thus, the avoidance of PPI medication may contribute to the prevention of dementia.”

# Threaten microbial diversity

- Antibiotics
- Method of delivery
- Medications
- Water treatment
- Diet
- Stress
- GMO

leading to increased gut permeability

ARTICLE

# **Strategies for overweight obese and normal weight adults with type 2 diabetes mellitus**

Sujung Yoon<sup>1,2</sup>, Hanbyul Cho<sup>3</sup>, Jungyoon Kim<sup>1,2</sup>, Do-Wan Lee<sup>1</sup>, Geon Ha Kim<sup>1</sup>, Young Sun Hong<sup>4</sup>, Sohyeon Moon<sup>1,5</sup>, Shinwon Park<sup>1,2</sup>, Sunho Lee<sup>1,6</sup>, Suji Lee<sup>1,2</sup>, Sujin Bae<sup>7</sup>, Donald C. Simonson<sup>8</sup>, In Kyoon Lyoo<sup>1,2,5</sup>

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### **Abstract**

**Aims/hypothesis** Overweight and obesity may significantly worsen glycaemic and metabolic control in type 2 diabetes. However, little is known about the effects of overweight and obesity on the brains of people with type 2 diabetes. Here, we investigate whether the presence of overweight or obesity influences the brain and cognitive functions during early stage type 2 diabetes.

**Methods** This study attempted to uncouple the effects of overweight/obesity from those of type 2 diabetes on brain

Suhng Yoon and Hanbyul Cho contributed equally to this study.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00125-017-4266-7) contains peer-reviewed but unedited supplementary material, which is available to authorized users.

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<sup>3</sup> Graduate School of Pharmaceutical Sciences, Ewha Womans University, Seoul, South Korea

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<sup>7</sup> Department of Psychiatry, Chung Ang University Hospital, Seoul, South Korea

structures and cognition. Overweight/obese participants with type 2 diabetes had more severe and progressive abnormalities in their brain structures and cognition during early stage type 2 diabetes compared with participants with normal weight. Relationships between each of these measures and disease duration were also examined.

*Results* Global mean cortical thickness was lower in the overweight/obese type 2 diabetes group than in the normal-weight type 2 diabetes group ( $z = -2.96$ ,  $p$  for group effect = 0.003). A negative correlation was observed between disease duration and global mean white matter integrity ( $z = 2.42$ ,  $p$  for interaction = 0.02) in the overweight/obese type 2 diabetes group, but not in the normal-weight type 2 diabetes group. Overweight/obese individuals with type 2 diabetes showed a decrease in psychomotor speed performance related to disease duration ( $z = -2.12$ ,  $p$  for interaction = 0.03), while normal-weight participants did not.

**Conclusions/interpretation** The current study attempted to uncouple the effects of overweight/obesity from those of type 2 diabetes on brain structures and cognition. Overweight/obese participants with type 2 diabetes had more severe and progressive abnormalities in brain structures and cognition during early stage type 2 diabetes compared with normal-weight participants.

**Keywords** Cognitive function . Grey matter . Obesity . Overweight . Type 2 diabetes mellitus . White matter

## Abbreviations

- |        |                                     |
|--------|-------------------------------------|
| FA     | Fractional anisotropy               |
| Hs-CRP | High-sensitivity C-reactive protein |
| ICV    | Intracranial volume                 |
| ROI    | Region of interest                  |

# Brain changes in overweight/obese and normal-weight adults

# Brain changes in overweight/obese and normal-weight adults

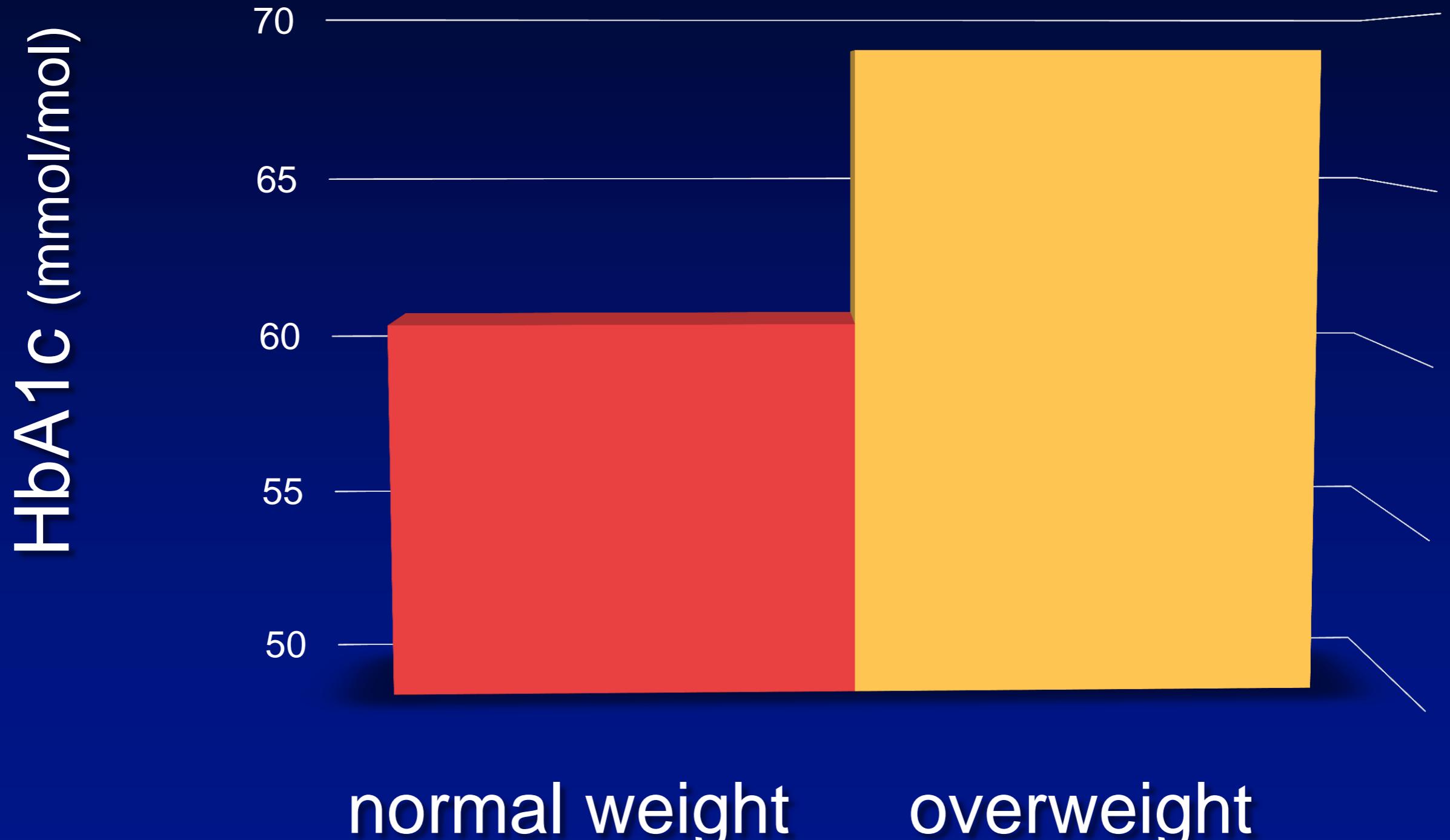
- 50 overweight/obese type 2 diabetics
- 50 normal-weight type 2 diabetics
- 50 normal-weight non-diabetics

# Brain changes in overweight/obese and normal-weight adul

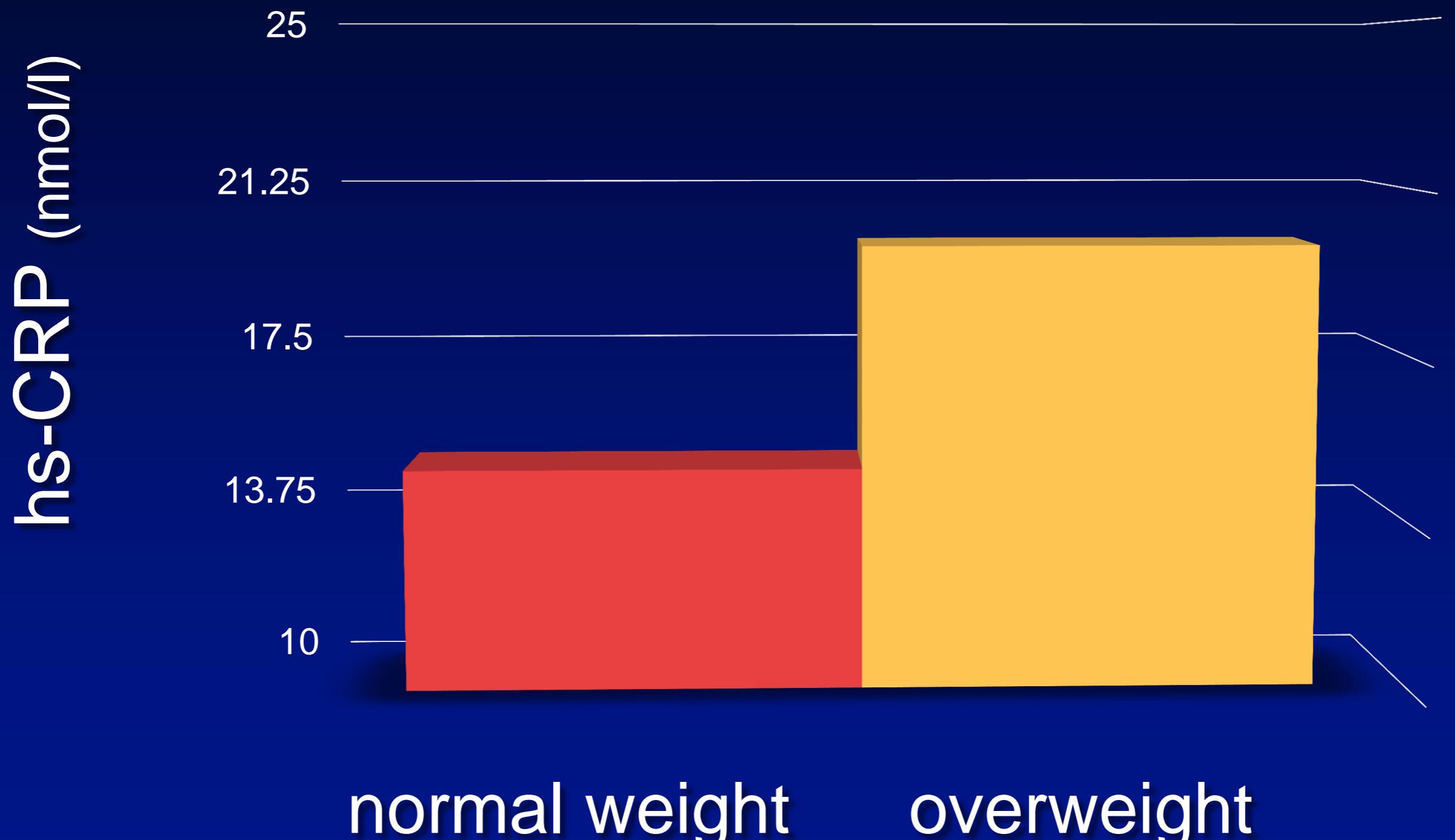
- glycemic parameters, renal functions, HOMA-IR, hs-CRP
- MRI brain imaging
- Cognitive assessment

Initially and at 1 year

# Type 2 Diabetics

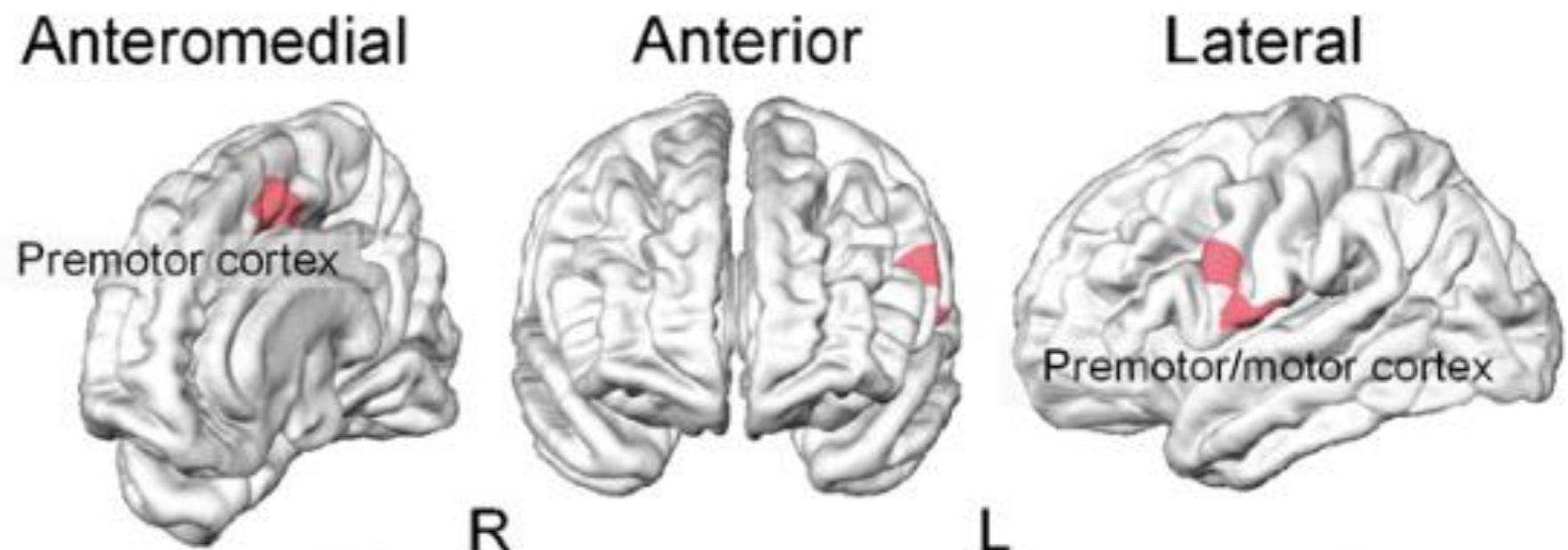


# Type 2 Diabetics

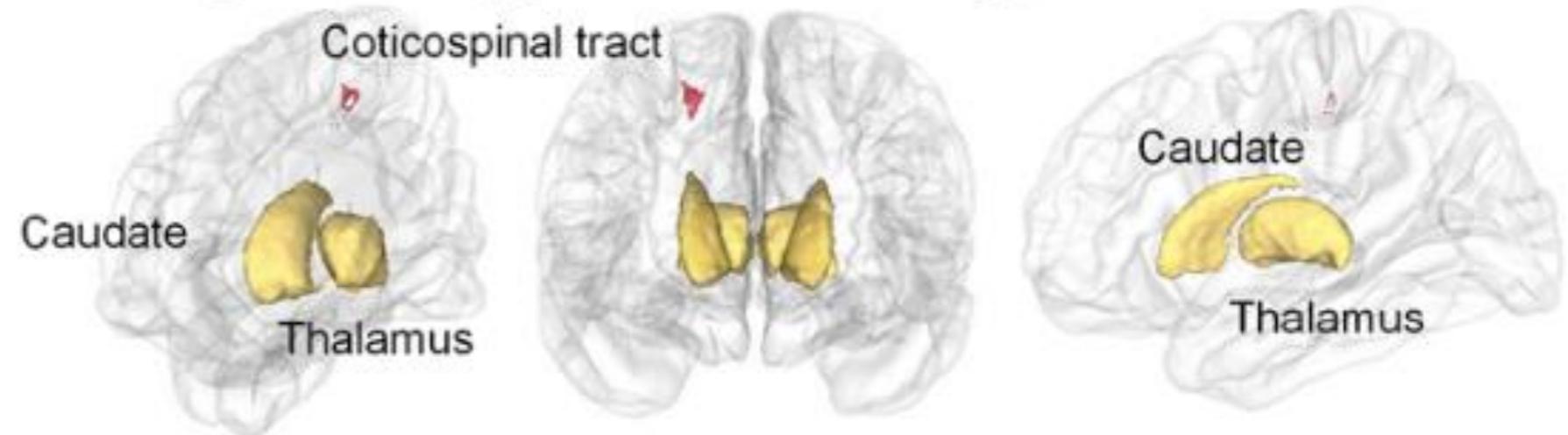


# Comparing region of interest (ROI) T2D normal vs overweight

Grey matter  
ROI  
(cortical thickness)

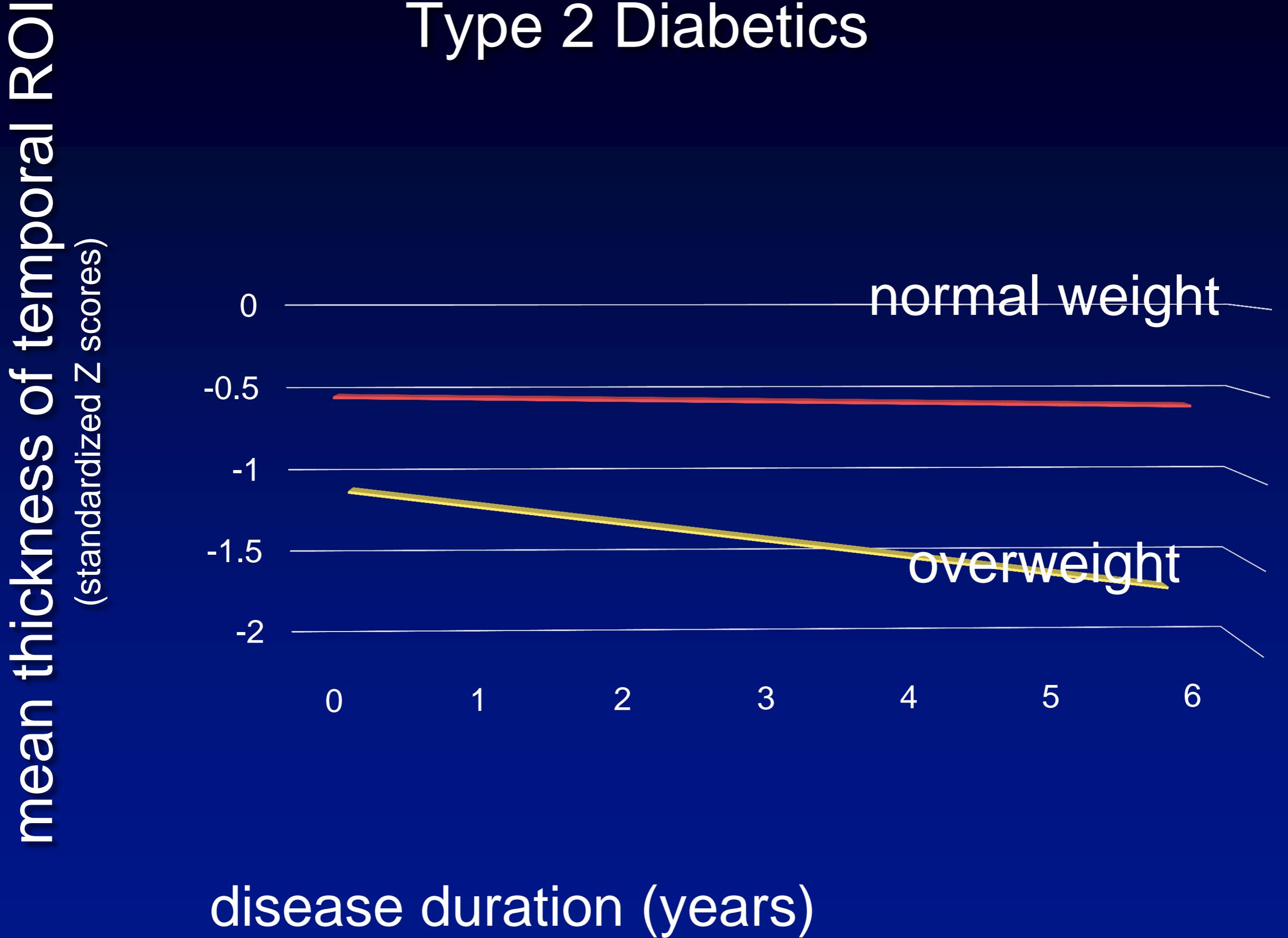


White matter  
ROI  
(FA values)

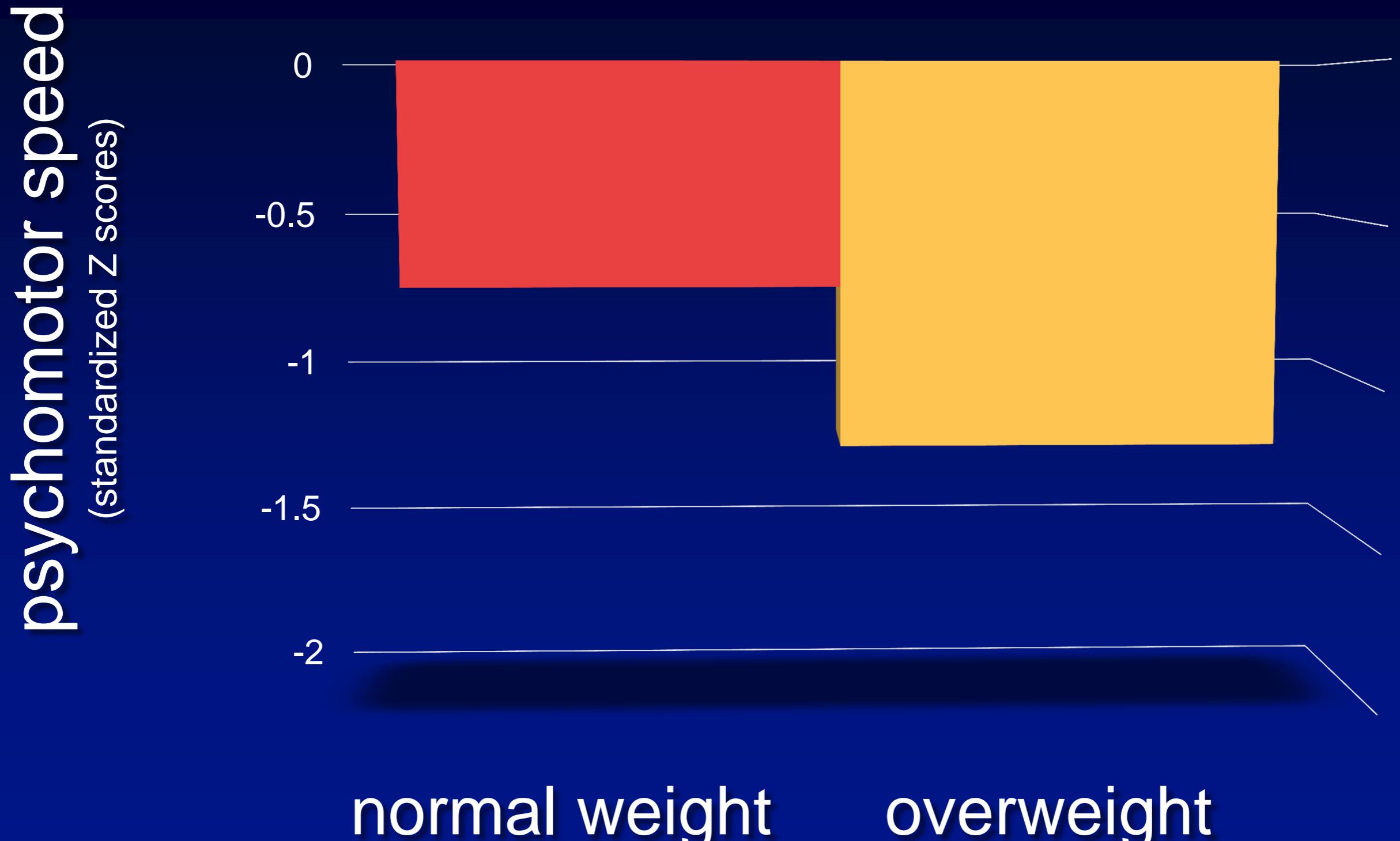


(FA vs A<sub>E</sub>)  
ROI

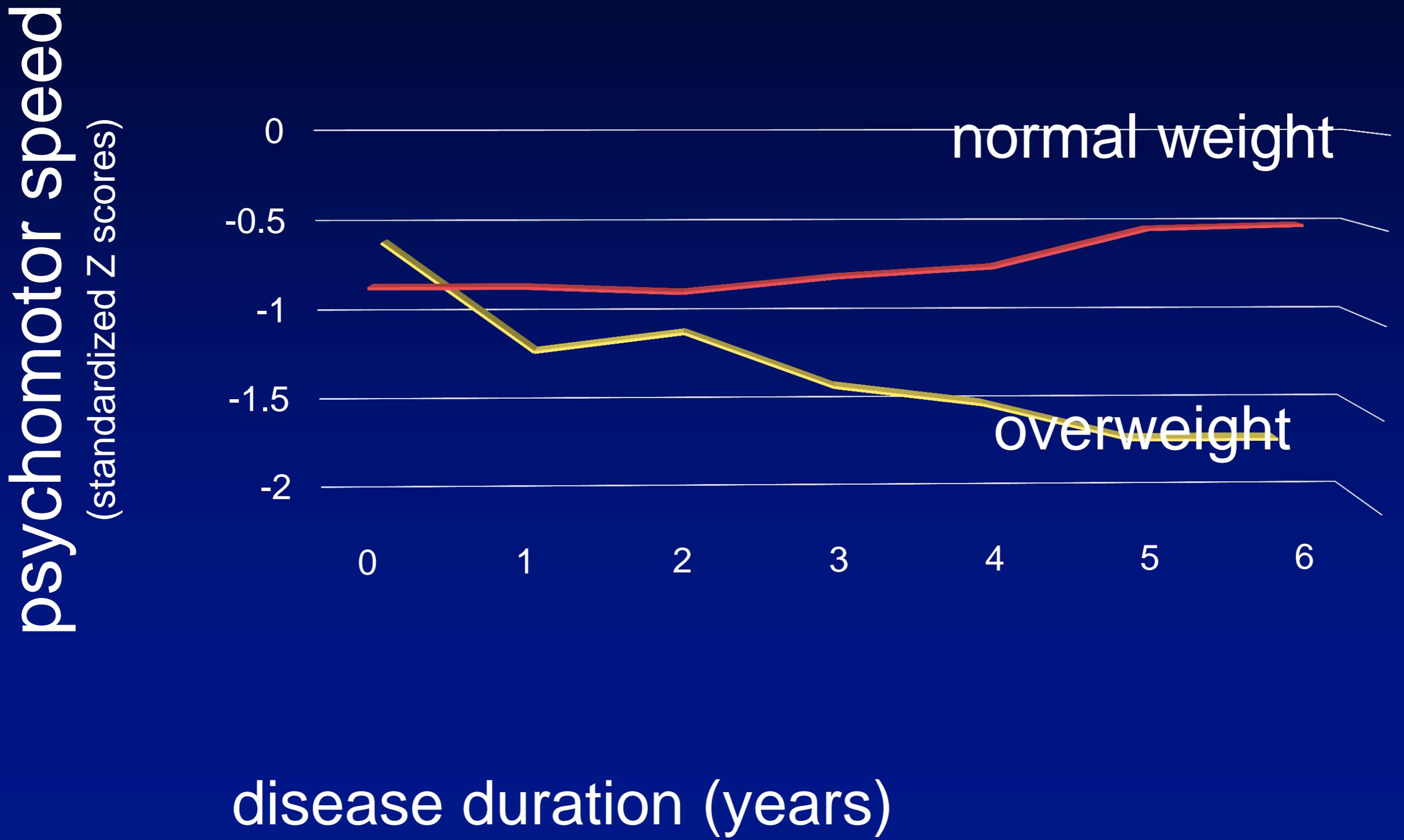
# Type 2 Diabetics



# Type 2 Diabetics



# Type 2 Diabetics



# Type 2 Diabetics



## Brain changes in overweight/obese and normal-weight adul

“This study showed that the concurrent presence of overweight/obesity was associated with cortical atrophy, disrupted white matter integrity and cognitive dysfunction in early stage type 2 diabetes. An increased awareness of overweight/obesity-related risk is necessary to prevent and manage type 2 diabetes-related brain atrophy and cognitive dysfunction from early stage type 2 diabetes onward.”

# How *not* to become diabetic

?



# Consumption of artificially and sugar-sweetened beverages and incident type 2 diabetes in the Etude Épidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale—European Prospective Investigation into Cancer and Nutrition cohort<sup>1–4</sup>

Guy Fagherazzi, Alice Vilier, Daniela Saes Sartorelli, Martin Lajous, Beverley Balkau, and Françoise Clavel-Chapelon

## ABSTRACT

**Background:** It has been extensively shown, mainly in US populations, that sugar-sweetened beverages (SSBs) are associated with increased risk of type 2 diabetes (T2D), but less is known about the effects of artificially sweetened beverages (ASBs).

**Objective:** We evaluated the association between self-reported SSB, ASB, and 100% fruit juice consumption and T2D risk over 14 y of follow-up in the French prospective Etude Épidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale—European Prospective Investigation into Cancer and Nutrition cohort.

**Design:** A total of 66,118 women were followed from 1993, and 1369 incident cases of T2D were diagnosed during the follow-up. Cox regression models were used to estimate HRs and 95% CIs for T2D risk.

**Results:** The average consumption of sweetened beverages in consumers was 328 and 568 mL/wk for SSBs and ASBs, respectively. Compared with nonconsumers, women in the highest quartiles of SSB and ASB consumers were at increased risk of T2D with HRs (95% CIs) of 1.34 (1.05, 1.71) and 2.21 (1.56, 3.14) for women who consumed >359 and >603 mL/wk of SSBs and ASBs, respectively. Strong positive trends in T2D risk were also observed across quartiles of consumption for both types of beverage ( $P = 0.0088$  and  $P < 0.0001$ , respectively). In sensitivity analyses, associations were partly mediated by BMI, although there was still a strong significant independent effect. No association was observed for 100% fruit juice consumption.

**Conclusions:** Both SSB consumption and ASB consumption were associated with increased T2D risk. We cannot rule out that factors other than ASB consumption that we did not control for are responsible for the association with diabetes, and randomized trials are required to prove a causal link between ASB consumption and T2D. *Am J Clin Nutr* 2013;97:517–23.

## INTRODUCTION

The consumption of sugar-sweetened beverages (SSBs)<sup>5</sup> has been extensively associated with increased risk of type 2 diabetes (T2D) (1) but also with weight gain (2), obesity (3), metabolic syndrome (1), hypertriglyceridemia (4, 5), coronary artery disease (6), and high blood pressure (7). These associations have been attributed to several potential mechanisms as follows: an incomplete compensatory reduction in energy intake

at subsequent meals after the intake of liquid calories (8), a glycemic effect with a rapid spike in blood glucose and insulin concentrations (9, 10), which could lead to insulin resistance over time, and a rapid hunger response or a harmful role of fructose (3). In addition, previous studies showed increased risk of T2D related to fruit juice consumption (11, 12), and mechanisms invoked were the same as for the association between SSBs and T2D risk.

In contrast, results for artificially sweetened beverages (ASBs) have been sparse and inconsistent, with some studies that showed increased risk of T2D, weight gain, and cardiometabolic dysfunction (2, 4, 5, 13–15). For instance, Schulze et al (2) failed to find a significant association between ASB consumption and T2D risk. In contrast, Nettleton et al (5) showed a significant association between both T2D and metabolic syndrome risk and ASB consumption, but the significance was lost after adjustment for BMI, which suggested that BMI is an intermediate factor. To

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<sup>2</sup> Study sponsors had no role in the design of the study, the analysis or interpretation of data, the writing of the manuscript, or the decision to submit the manuscript for publication.

<sup>3</sup> Supported by the Institut National du Cancer, the Mutuelle Générale de l'Education Nationale, the Institut de Cancérologie Gustave Roussy, and the Institut National de la Santé et de la Recherche Médicale. The validation of potential diabetes cases was supported by the European Union (Integrated Project LSHM-CT-2006-037197 in the Framework Programme 6 of the European Community) InterAct project.

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<sup>5</sup> Abbreviations used: ASB, artificially sweetened beverage; E3N, Etude Épidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale; SSB, sugar-sweetened beverage; T2D, type 2 diabetes.

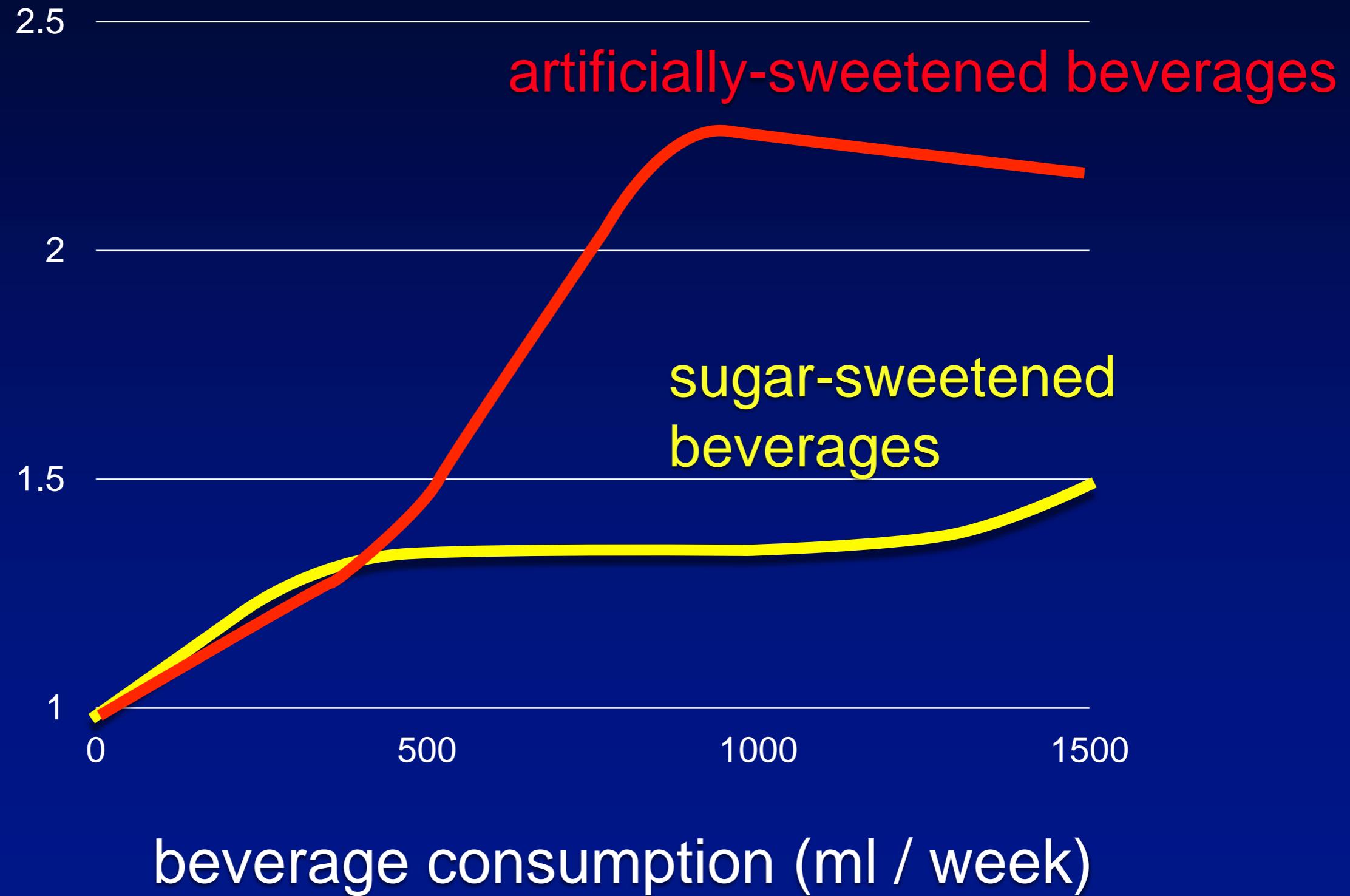
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# Consumption of artificially and sugar-sweetened beverages and incident type 2 diabetes

- 66,118 women followed 14 years
- Consumption of sugar-sweetened beverages, artificially-sweetened beverages
- Incident cases of type 2 DM

risk for type 2 diabetes



# **ARTIFICIAL SWEETENERS: POWERFUL LINK TO DIABETES**



**Artificial Sweeteners Threaten Your Health**

**Artificial Sweeteners Threaten Your Health**

# Artificial sweeteners induce glucose intolerance by altering the gut microbiota

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Non-caloric artificial sweeteners (NAS) are among the most widely used food additives worldwide, regularly consumed by lean and obese individuals alike. NAS consumption is considered safe and beneficial owing to their low caloric content, yet supporting scientific data remain sparse and controversial. Here we demonstrate that consumption of commonly used NAS formulations drives the development of glucose intolerance through induction of compositional and functional alterations to the intestinal microbiota. These NAS-mediated deleterious metabolic effects are abrogated by antibiotic treatment, and are fully transferrable to germ-free mice upon faecal transplantation of microbiota configurations from NAS-consuming mice, or of microbiota anaerobically incubated in the presence of NAS. We identify NAS-altered microbial metabolic pathways that are linked to host susceptibility to metabolic disease, and demonstrate similar NAS-induced dysbiosis and glucose intolerance in healthy human subjects. Collectively, our results link NAS consumption, dysbiosis and metabolic abnormalities, thereby calling for a reassessment of massive NAS usage.

Non-caloric artificial sweeteners (NAS) were introduced over a century ago as means for providing sweet taste to foods without the associated high energy content of caloric sugars. NAS consumption gained much popularity owing to their reduced costs, low caloric intake and perceived health benefits for weight reduction and normalization of blood sugar levels<sup>1</sup>. For these reasons, NAS are increasingly introduced into commonly consumed foods such as diet sodas, cereals and sugar-free desserts, and are being recommended for weight loss and for individuals suffering from glucose intolerance and type 2 diabetes mellitus<sup>1</sup>.

Some studies showed benefits for NAS consumption<sup>2</sup> and little induction of a glycaemic response<sup>3</sup>, whereas others demonstrated associations between NAS consumption and weight gain<sup>4</sup>, and increased type 2 diabetes risk<sup>5</sup>. However, interpretation is complicated by the fact that NAS are typically consumed by individuals already suffering from metabolic syndrome manifestations. Despite these controversial data, the US Food and Drug Administration (FDA) approved six NAS products for use in the United States.

Most NAS pass through the human gastrointestinal tract without being digested by the host<sup>6,7</sup> and thus directly encounter the intestinal microbiota, which plays central roles in regulating multiple physiological processes<sup>8</sup>. Microbiota composition<sup>9</sup> and function<sup>10</sup> are modulated by diet in the healthy/lean state as well as in obesity<sup>11,12</sup> and diabetes mellitus<sup>13</sup>, and in turn microbiota alterations have been associated with propensity to metabolic syndrome<sup>14</sup>. Here, we study NAS-mediated modulation of microbiota composition and function, and the resultant effects on host glucose metabolism.

## Chronic NAS consumption exacerbates glucose intolerance

To determine the effects of NAS on glucose homeostasis, we added commercial formulations of saccharin, sucralose or aspartame to the

drinking water of lean 10-week-old C57BL/6 mice (Extended Data Fig. 1a). Since all three commercial NAS comprise ~5% sweetener and ~95% glucose, we used as controls mice drinking only water or water supplemented with either glucose or sucrose. Notably, at week 11, the three mouse groups that consumed water, glucose and sucrose featured comparable glucose tolerance curves, whereas all three NAS-consuming mouse groups developed marked glucose intolerance ( $P < 0.001$ , Fig. 1a, b).

As saccharin exerted the most pronounced effect, we further studied its role as a prototypical artificial sweetener. To corroborate the findings in the obesity setup, we fed C57BL/6 mice a high-fat diet (HFD, 60% kcal from fat) while consuming either commercial saccharin or pure glucose as a control (Extended Data Fig. 1b). As in the lean state, mice fed HFD and commercial saccharin developed glucose intolerance, compared to the control mouse group ( $P < 0.03$ , Fig. 1c and Extended Data Fig. 2a). To examine the effects of pure saccharin on glucose intolerance, we followed a cohort of 10-week-old C57BL/6 mice fed on HFD and supplemented with  $0.1 \text{ mg ml}^{-1}$  of pure saccharin added to their drinking water (Extended Data Fig. 1c). This dose corresponds to the FDA acceptable daily intake (ADI) in humans (5 mg per kg (body weight), adjusted to mouse weights, see Methods). As with commercial saccharin, this lower dose of pure saccharin was associated with impaired glucose tolerance ( $P < 0.0002$ , Fig. 1d and Extended Data Fig. 2b) starting as early as 5 weeks after HFD initiation. Similarly, HFD-fed outbred Swiss Webster mice supplemented with or without  $0.1 \text{ mg ml}^{-1}$  of pure saccharin (Extended Data Fig. 1d) showed significant glucose intolerance after 5 weeks of saccharin exposure as compared to controls ( $P < 0.03$ , Extended Data Fig. 2c, d).

Metabolic profiling of normal-chow- or HFD-fed mice in metabolic cages, including liquids and chow consumption, oxygen consumption, walking distance and energy expenditure, showed similar measures between NAS- and control-drinking mice (Extended Data Fig. 3 and 4).

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haemoglobin (HbA1C%) and glucose tolerance test (GTT, measures of impaired glucose tolerance), and elevated serum alanine aminotransferase (ALT, measure of hepatic damage that is likely to be secondary, in this context, to non-alcoholic fatty liver disease). Moreover, the levels of glycosylated haemoglobin (HbA1C%), indicative of glucose concentration over the previous 3 months, were significantly increased when comparing a subgroup of high-NAS consumers (40 individuals) to non-NAS consumers (236 individuals, Fig. 4a, rank sum  $P < 0.002$ ). This increase remained significant when corrected to body mass index (BMI) levels (rank sum  $P < 0.015$ ). In this cohort, we characterized the 16S rRNA in 172 randomly selected individuals. Notably, we found statistically significant positive correlations between multiple taxonomic entities and NAS consumption, including the *Enterobacteriaceae* family (Pearson  $r = 0.36$ , FDR corrected  $P < 10^{-6}$ ), the Deltaproteobacteria class (Pearson  $r = 0.33$ , FDR corrected  $P < 10^{-5}$ ) and the Actinobacteria phylum (Pearson  $r = 0.27$ , FDR corrected  $P < 0.0003$ , Supplementary Table 7). Importantly, we did not detect statistically significant correlations between OTU abundances and BMI, suggesting that the above correlations are not due to the distinct BMI of NAS consumers.

Finally, as an initial assessment of whether the relationship between human NAS consumption and blood glucose control is causative, we followed seven healthy volunteers (5 males and 2 females, aged 28–36) who do not normally consume NAS or NAS-containing foods for 1 week. During this week, participants consumed on days 2–7 the FDA's maximal acceptable daily intake (ADI) of commercial saccharin (5 mg per kg (body weight)) as three divided daily doses equivalent to 120 mg, and were monitored by continuous glucose measurements and daily GTT (Extended Data Fig. 9a). Notably, even in this short-term 7-day exposure period, most individuals (4 out of 7) developed significantly poorer glycaemic responses 5–7 days after NAS consumption (hereafter termed 'NAS responders'), compared to their individual glycaemic response on days 1–4 (Fig. 4b, c and Extended Data Fig. 9b,  $P < 0.001$ ). None of the three NAS non-responders featured improved glucose tolerance (Fig. 4b, d and Extended Data Fig. 9c).

The microbiome configurations of NAS responders, as assessed by 16S rRNA analysis, clustered differently from non-responders both before and after NAS consumption (Fig. 4e and Extended Data Fig. 9d, respectively). Moreover, microbiomes from non-responders featured little changes in composition during the study week, whereas pronounced compositional changes were observed in NAS responders (Fig. 4f and Extended Data Fig. 9e). To study whether this NAS-induced dysbiosis has a causal role in generating glucose intolerance, stool from before (day 1, D1) or after (day 7, D7) NAS exposure were transferred from two NAS responders and two NAS non-responders into groups of normal-chow-fed germ-free mice. Indeed, transfer of post-NAS exposure (D7) stool from NAS responders induced significant glucose intolerance in recipient germ-free mice, compared to the response noted with D1 stool transferred from the same NAS-responder individuals (Fig. 4g and Extended Data Fig. 9f,  $P < 0.004$  and Extended Data Fig. 9g, h,  $P < 0.02$ ). In contrast, D7 stools transferred into germ-free mice from the two NAS non-responders induced normal glucose tolerance, which was indistinguishable from that of mice transferred with D1 stools from the same 'non-responding' individuals (Fig. 4h and Extended Data Fig. 9i–k). Germ-free mice transplanted with 'responders' microbiome replicated some of the donor saccharin-induced dysbiosis, including 20-fold relative increase of *Bacteroides fragilis* (order Bacteroidales) and *Weissella cibaria* (order Lactobacillales), and approximately tenfold decrease in *Candidatus Arthromitus* (order Clostridiales) (Extended Data Fig. 9l).

## Discussion

In summary, our results suggest that non-caloric artificial sweetener consumption in both mice and humans enhances the risk of glucose intolerance and that these adverse metabolic effects are mediated by modulation of the composition and function of the microbiota. *i*nated with type 2 diabetes in humans<sup>1–4</sup>, including over-representation

of *Bacteroides* and under-representation of *Clostridiales*. Both Gram-positive and Gram-negative taxa contributed to the NAS-induced phenotype (Fig. 1a, b) and were enriched for glycan degradation pathways (Extended Data Fig. 6), previously linked to enhanced energy harvest (Fig. 2c, d)<sup>11,24</sup>. This suggests that elaborate inter-species microbial cooperation may functionally orchestrate the gut ecosystem and contribute to vital community activities in diverging environmental conditions (for example, normal-chow versus high-fat dietary conditions). In addition, we show that metagenomes of saccharin-consuming mice are enriched with multiple additional pathways previously shown to associate with diabetes mellitus<sup>23</sup> or obesity<sup>11</sup> in mice and humans, including sphingolipid metabolism and lipopolysaccharide biosynthesis<sup>25</sup>.

Our results from short- and long-term human NAS consumer cohorts (Fig. 4, Extended Data Fig. 9 and Supplementary Tables 6, 7) suggest that human individuals feature a personalized response to NAS, possibly stemming from differences in their microbiota composition and function. The changes noted in our studies may be further substantiated in mice consuming different human diets<sup>26</sup>. Similarly, we believe that other individualized nutritional responses may be driven by personalized functional differences in the microbiome. As such, 'personalized nutrition' leading to 'personalized medical outcome' may underlie the variable nutritional effects noted in many multi-factorial diseases, and warrants further studies.

Artificial sweeteners were extensively introduced into our diets with the intention of reducing caloric intake and normalizing blood glucose levels without compromising the human 'sweet tooth'. Together with other major shifts that occurred in human nutrition, this increase in NAS consumption coincides with the dramatic increase in the obesity and diabetes epidemics. Our findings suggest that NAS may have directly contributed to enhancing the exact epidemic that they themselves were intended to fight. Moreover, our results point towards the need to develop new nutritional strategies tailored to the individual while integrating personalized differences in the composition and function of the gut microbiota.

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Finally, as an initial assessment of whether the relationship between human NAS consumption and blood glucose control is causative, we followed seven healthy volunteers (5 males and 2 females, aged 28–36) who do not normally consume NAS or NAS-containing foods for 1 week. During this week, participants consumed on days 2–7 the FDA's maximal acceptable daily intake (ADI) of commercial saccharin (5 mg per kg (body weight)) as three divided daily doses equivalent to 120 mg, and were monitored by continuous glucose measurements and daily GTT (Extended Data Fig. 9a). Notably, even in this short-term 7-day exposure period, most individuals (4 out of 7) developed significantly poorer glycaemic responses 5–7 days after NAS consumption (hereafter termed 'NAS responders'), compared to their individual glycaemic response on days 1–4 (Fig. 4b, c and Extended Data Fig. 9b,  $P < 0.001$ ). None of the three NAS non-responders featured improved glucose tolerance (Fig. 4b, d and Extended Data Fig. 9c).

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## Discussion

In summary, our results suggest that NAS consumption in both mice and humans enhances the risk of glucose intolerance and that these adverse metabolic effects are mediated by modulation of the composition and function of the microbiota. Notably, several of the bacterial taxa that changed following NAS consumption were previously associated with type 2 diabetes in humans<sup>19,20</sup>, including over-representation

of *Bacteroides* and under-representation of *Clostridiales*. Both Gram-positive and Gram-negative taxa contributed to the NAS-induced phenotype (Fig. 1a, b) and were enriched for glycan degradation pathways (Extended Data Fig. 6), previously linked to enhanced energy harvest (Fig. 2c, d)<sup>11,24</sup>. This suggests that elaborate inter-species microbial cooperation may functionally orchestrate the gut ecosystem and contribute to vital community activities in diverging environmental conditions (for example, normal-chow versus high-fat dietary conditions). In addition, we show that metagenomes of saccharin-consuming mice are enriched with multiple additional pathways previously shown to associate with diabetes mellitus<sup>23</sup> or obesity<sup>11</sup> in mice and humans, including sphingolipid metabolism and lipopolysaccharide biosynthesis<sup>25</sup>.

Our results from short- and long-term human NAS consumer cohorts (Fig. 4, Extended Data Fig. 9 and Supplementary Tables 6, 7) suggest that human individuals feature a personalized response to NAS, possibly stemming from differences in their microbiota composition and function. The changes noted in our studies may be further substantiated in mice consuming different human diets<sup>26</sup>. Similarly, we believe that other individualized nutritional responses may be driven by personalized functional differences in the microbiome. As such, 'personalized nutrition' leading to 'personalized medical outcome' may underlie the variable nutritional effects noted in many multi-factorial diseases, and warrants further studies.

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## Original Contribution

# Sugar- and Artificially Sweetened Beverages and the Risks of Incident Stroke and Dementia: A Prospective Cohort Study

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Claudia L. Satizabal, PhD; Ramachandran S. Vasan, MD; Sudha Seshadri, MD\*; Paul F. Jacques, DSc\*

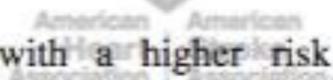
**Background and Purpose**—Sugar- and artificially-sweetened beverage intake have been linked to cardiometabolic risk factors, which increase the risk of cerebrovascular disease and dementia. We examined whether sugar- or artificially sweetened beverage consumption was associated with the prospective risks of incident stroke or dementia in the community-based Framingham Heart Study Offspring cohort.

**Methods**—We studied 2888 participants aged >45 years for incident stroke (mean age 62 [SD, 9] years; 45% men) and 1484 participants aged >60 years for incident dementia (mean age 69 [SD, 6] years; 46% men). Beverage intake was quantified using a food-frequency questionnaire at cohort examinations 5 (1991–1995), 6 (1995–1998), and 7 (1998–2001). We quantified recent consumption at examination 7 and cumulative consumption by averaging across examinations. Surveillance for incident events commenced at examination 7 and continued for 10 years. We observed 97 cases of incident stroke (82 ischemic) and 81 cases of incident dementia (63 consistent with Alzheimer's disease).

**Results**—After adjustments for age, sex, education (for analysis of dementia), caloric intake, diet quality, physical activity, and smoking, higher recent and higher cumulative intake of artificially sweetened soft drinks were associated with an increased risk of ischemic stroke, all-cause dementia, and Alzheimer's disease dementia. When comparing daily cumulative intake to 0 per week (reference), the hazard ratios were 2.96 (95% confidence interval, 1.26–6.97) for ischemic stroke and 2.89 (95% confidence interval, 1.18–7.07) for Alzheimer's disease. Sugar-sweetened beverages were not associated with stroke or dementia.

**Conclusions**—Artificially sweetened soft drink consumption was associated with a higher risk of stroke and dementia. (*Stroke*. 2017;48:00-00. DOI: 10.1161/STROKEAHA.116.016027.)

**Key Words:** dementia ■ Framingham Heart Study ■ soft drinks ■ stroke ■ sugar



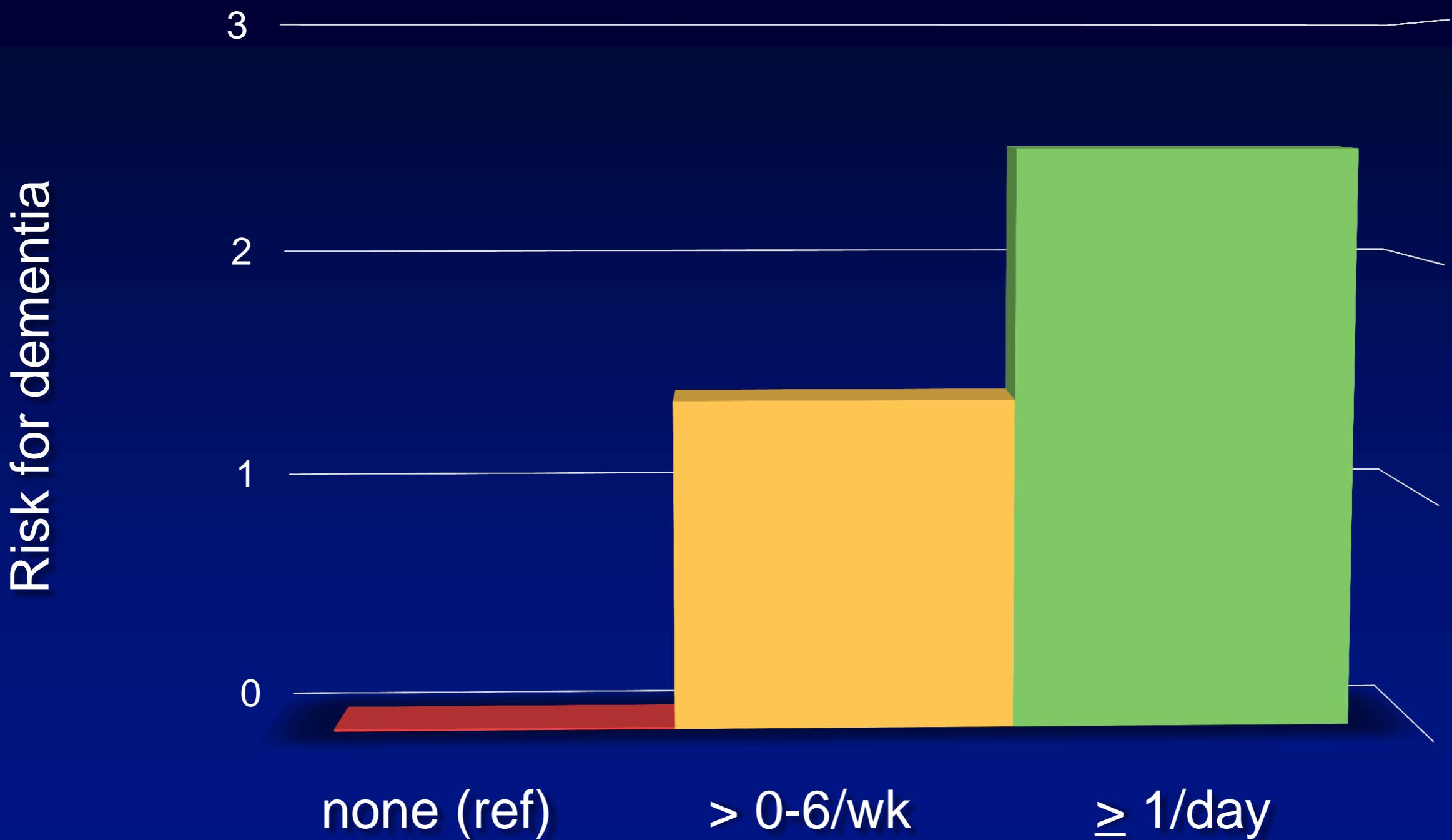
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# Sugar- and Artificially Sweetened Beverages and the Risks of Incident Stroke and Dementia A Prospective Cohort Study

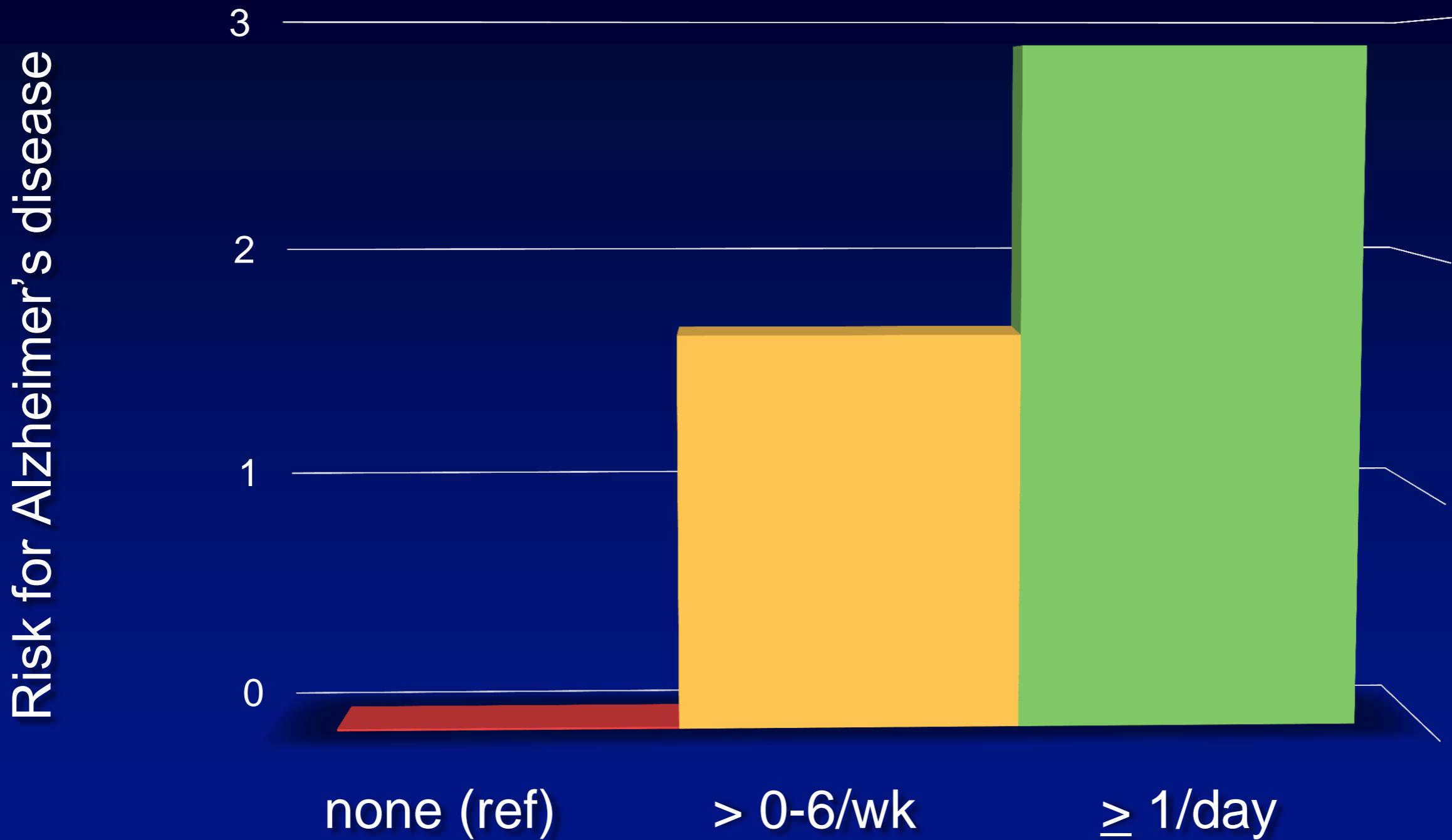
# Sugar- and Artificially Sweetened Beverages and the Risks of Incident Stroke and Dementia A Prospective Cohort Study

- 2888 > 45 years studied for stroke
- 1484 > 60 years studied for dementia
- Food frequency questionnaire for artificially sweetened soft drinks between 1991 and 2001
- Adjust for age, physical activity, smoking, gender, education, caloric intake, diet quality

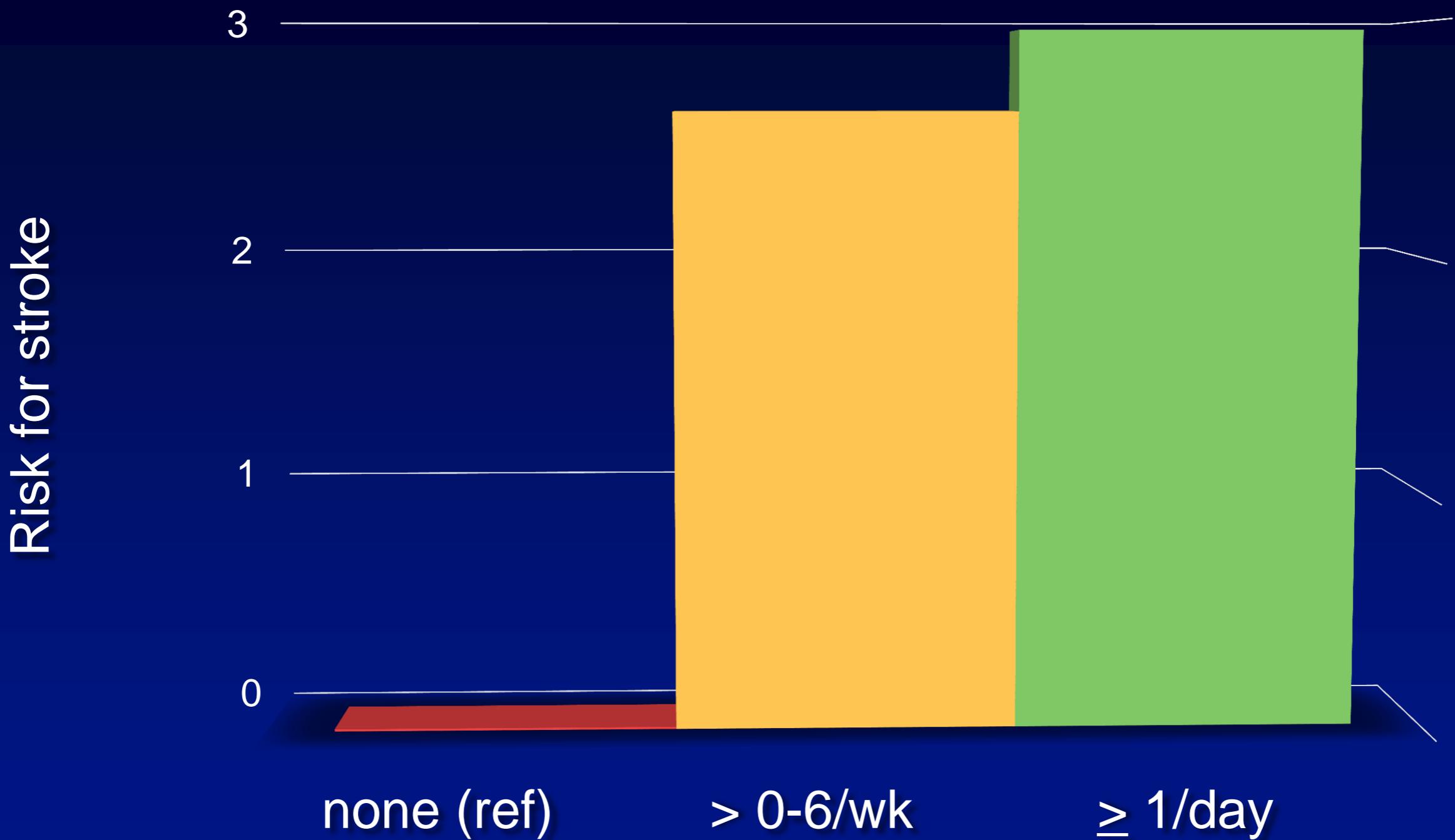
# Artificially sweetened soft drinks and dementia risk



# Artificially sweetened soft drinks and Alzheimer's risk



# Artificially sweetened soft drinks risk for ischemic stroke



# Sugar- and Artificially Sweetened Beverages and the Risks of Incident Stroke and Dementia A Prospective Cohort Study

“Artificial sweeteners have been shown to cause glucose intolerance in mice by altering gut microbiota and are associated with dysbiosis and glucose intolerance in humans.”

# **ARTIFICIAL SWEETENERS: POWERFUL LINK TO ALZHEIMER'S**



**Artificial Sweeteners Threaten Your Health**

**Artificial Sweeteners Threaten Your Health**

# Leveraging Lifestyle for Brain Health

David Perlmutter, MD  
[DrPerlmutter.com](http://DrPerlmutter.com)

