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Why Clinical Trials Show that Strict Glucose Regulation Does *Not* prevent Diabetic Complications in T2D: Evidence Supporting an Alternative Hypothesis for Pathogenesis

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Keynote Overview

- What is at stake?
- Classic hyperglycemia hypothesis (HH) for pathogenesis of diabetic complications.
- Clinical trials testing HH in T1D and T2D
- Alternative insulin-IGF Hypothesis
- Tests of insulin-IGF hypothesis in diabetic peripheral neuropathy
- Insulin and insulin-like growth factors (IGFs) are neurotrophic factors
- Deficiency in insulin/IGF axis in diabetes
- Increased risk for brain atrophy, impaired cognition and Alzheimer's in diabetes

Keynote Overview (cont.)

- Tests of insulin/IGF hypothesis in diabetic brain atrophy
- IGFs prevent impaired cognition in diabetes
- Insulin and IGFs regulate total brain protein levels and prevent brain atrophy in diabetes independently of hyperglycemia
- Hyperglycemia is NOT the primary cause of diabetic neurological complications. Nor are secondary consequences of hyperglycemia such as polyols, protein glycation or advanced glycation end products (AGE).
- Why insulin partially works in T1D trials, whereas intensive hyperglycemia therapy fails in T2D trials
- Many anomalies to HH now explained
- Conclusions and Recommendations

What Is At Stake?

- **Worldwide, 415 million people have diabetes.**
- **US $673 billion cost for care of diabetes, and disproportionately towards care of complications**
- **Worldwide, 46 million people have Alzheimer's**
- **US $818 billion cost for care of dementia.**

- **The national health care budget of many nations is at risk should effective new treatments fail to emerge soon.**
- **A new hypothesis for pathogenesis is needed to guide the development of rational therapeutic treatments.**

Classic Hyperglycemia Hypothesis (HH)

- **Insulin was discovered to reduce hyperglycemia and stabilize T1D patients (Banting, Best, Collip, Macleod).**
- **Logical hypothesis: Hyperglycemia in diabetes is the cause of complications (neuropathy, retinopathy and nephropathy) (Greene et al., 1987; Brownlee et al., 1988; Brownlee, 1990; Nathan, 1994; Hill et al., 1996; others).**
  - **Increased polyols, AGE, RAGE**
  - **Increased enzymatic & nonenzymatic protein glycation**
  - **Glycation causes tissue damage and complications**
  - **Glycation causes microvascular damage, etc.**
- **HH has been the dominant hypothesis for many decades**
  - **Medical training**
  - **Basis for interpretation of hundreds of research papers**

Clinical Tests of Hyperglycemia Hypothesis

- **Intensive insulin therapy reduces complications in T1D.**
  - **But, why not in 25-40% non-responders? (DCCT, 1993)**
- **Aldose reductase inhibitors (block polyols), 32 failed trials**
- **Intensive anti-hyperglycemia therapy does *not* prevent neuropathy, retinopathy nor nephropathy in T2D.**
  - **Meta-analysis of >34,500 patients (Boussageon et al., 2011).**
  - **HH test fails in T2D comprising 90% of diabetic patients.**
- **Protein glycation maximal in weeks (consider protein turnover), whereas complications emerge only after years-to-decades. No correlation of HH with disease time-course.**
- **HH cannot explain the 9-year lag between diabetes onset and emergence of nephropathy, proliferative retinopathy or neuropathy (Krolewski et al, 1987; Palumbo et al, 1978).**

Alternative To Hyperglycemia Hypothesis

- **Loss of insulin and IGF activity is pathogenic for diabetic PNS and CNS complications, *not* hyperglycemia (Ishii, 1995).**
- **This alternative insulin/IGF hypothesis was tested first in diabetic peripheral neuropathy.**
- **Subsequently, this hypothesis was tested in diabetic CNS complications.**

Insulin and IGFs are neurotrophic factors

- IGFs support peripheral (motor, sensory and autonomic) neurons as well as CNS neurons.
- Insulin and IGF receptors are present on virtually all neurons and glia. Insulin and IGFs present in blood and CSF.
- Insulin and IGFs increase neurite outgrowth and survival in cultured neurons.
- IGF levels are increased in injured nerves, and are required for axon elongation, nerve terminal sprouting, synapse regeneration, and neuron survival in rodents.
- Anti-IGF antisera mimics syndrome of neuropathy in non-diabetic rodents.
- IGFs cross the BBB, and are required for learning and memory.
- Reviews: Recio-Pinto and Ishii, 1988; Ishii et al., 1994

Prediction 1: Insulin and IGF activities are diminished in diabetes

- T1D: insufficient insulin production
- T2D: insulin resistance and partial reduction in production
- IGF circulating levels normally decline with age (Tan & Baxter, 1986)
- IGF levels decline faster & further in T1D and T2D
- IGF mRNA levels reduced in peripheral nerves, brain, spinal cord and liver in diabetic rodents
- Insulin transport into CNS is impaired in insulin resistance
- IGF levels are reduced in CNS in diabetes
- Prediction 1 is validated

Tests of Alternate Hypothesis (cont.)

- **Prediction 2: Replacement of IGF can ameliorate or prevent diabetic peripheral neuropathy. Confirmed in rodents by multiple separate labs for:**
  - **Hyperalgesia**
  - **Impaired nerve regeneration (cause of dying-back axonopathy)**
  - **Neuraxonal dystrophy (Abnormal nerve ultrastructure)**
  - **Loss of cutaneous nerves**
  - **Impaired neurogenic wound healing**

Tests of Alternate Hypothesis (cont.)

- **Prediction 3: IGF treatment is effective despite unabated hyperglycemia. Confirmed by multiple labs.**
- **Prediction 4: Anti-hyperglycemia therapy is ineffective. (confirmed clinically 17 years later by meta-analysis)**
- **Review: Ishii and Lupien, 2003**

Diabetes is Closely Associated With Late-Onset Alzheimer's Disease (LOAD)

- LOAD (95% of AD cases) emerges after age 65 and does *not* involve mutations in APP, presenilin-1 or -2 genes.
- Among LOAD brains, 81% of cases had a prior history of T2D or IGT (Janson et al., 2004).
- LOAD is associated with insulin resistance and low IGF levels (many studies).
- Brain insulin levels are reduced in CSF of LOAD patients (Craft et al., 1998).
- At advancing stages of LOAD, there is a progressive loss of insulin, insulin R, IGF-I and IGF R mRNAs in the frontal lobes of autopsy brains (Rivera et al., 2005).
- MRI reveals brain atrophy in T1D and T2D
- Cognitive disturbances are present in T1D and T2D

CNS Research Goals

LOAD is due to a progressive loss of up to 1/3 brain mass.

Current Alzheimer's drugs are poorly effective because they do not prevent ongoing brain atrophy.

Blocking plaques and tangles does *not* prevent dementia nor time to death in LOAD (Elan clinical trial; Holmes et al., 2008).

Identical twin studies show that environment much more important than genes in determining onset of LOAD dementia/P&T: *onset can be delayed >20 years.*

Note that insulin/IGF axis is environmentally responsive.

The cause of adult brain atrophy is presently unknown

GOAL 1: Identify the biochemical cause of progressive adult brain atrophy.

GOAL 2: Test insulin/IGF hypothesis in CNS: whether cognitive deficits as well as brain atrophy can be prevented in diabetes irrespective of hyperglycemia.

Shared Features: LOAD, Clinical Diab, and Exptl Diab.

Parameter	LOAD	Clin Diab	Exptl Diab
Brain atrophy	+	+	+
Dementia	+	+, a	+
Reduced brain insulin signaling	+, b	+	+
Aging is a major risk factor	+	+	+
IGF levels reduced with aging	+	+	+
IGF levels reduced with disease	+, c	+	+

a, Risk of dementia increased independently of cerebrovascular disease.
b, Reduced CSF insulin levels and uptake into brain
c, IGF levels fall in aging and further in diabetes and LOAD.

Common factor: reduced brain insulin and IGF signaling.

Note: insulin does not regulate brain glucose utilization.

IGF Regulates Learning & Memory (L&M)

L&M is widely believed to be encoded in synapses.

IGFs increase nerve terminal sprouting during synaptogenesis

They support nerve regeneration and re-establishment of synapses.

They increase dendritic spine density in brain slices.

Elderly with low IGF levels do poorly on standard L&M exams.

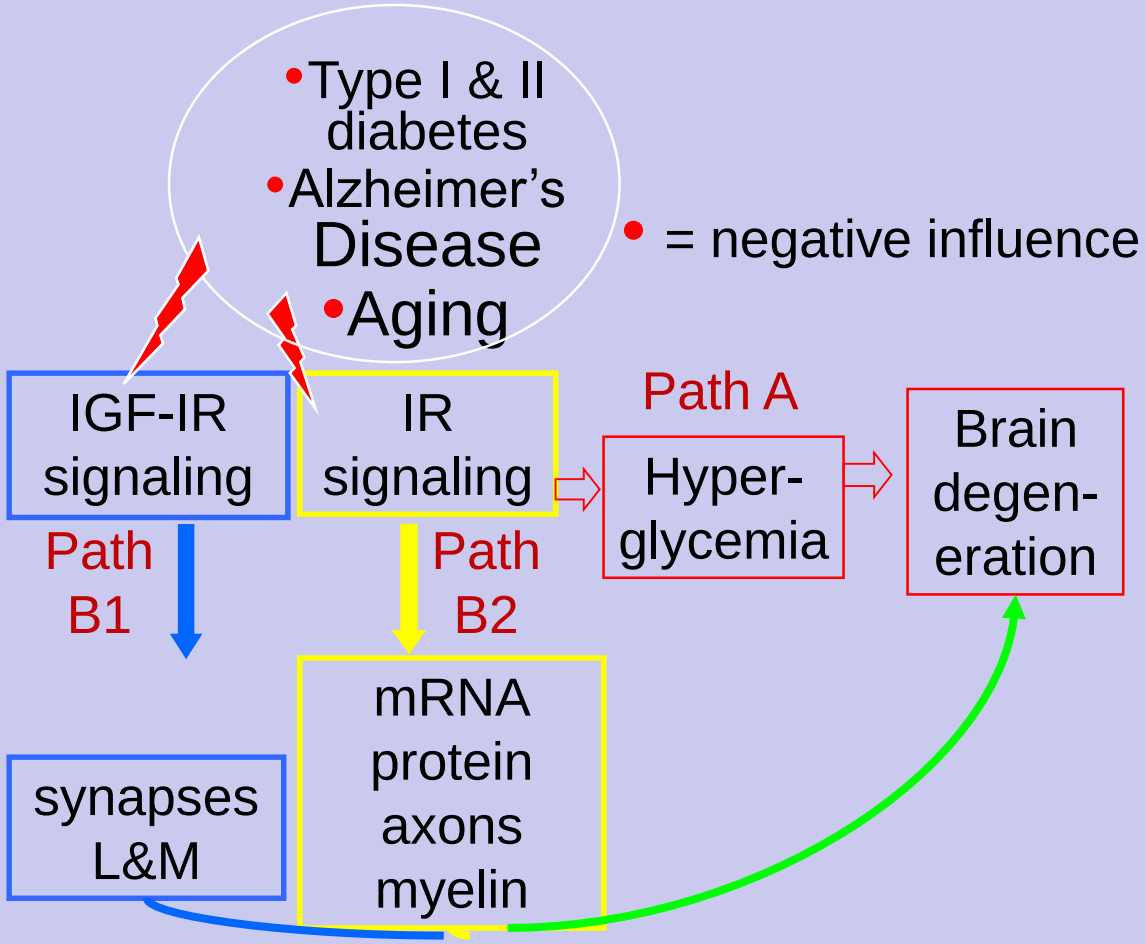
Diabetic and LOAD patients: low IGF levels and impaired L&M.

Test 1: Infuse anti-IGF antibodies i.c.v. in rats. This results in profound impairment of L&M. IGF is required for L&M.

Test 2: IGF replacement doses s.c. for 11 weeks in diabetic rats. IGF crosses BBB and prevents loss of L&M in Morris Water Maze despite hyperglycemia (Lupien et al., 2003).

Hypothesis: brain mass is regulated by the combination of insulin and IGFs

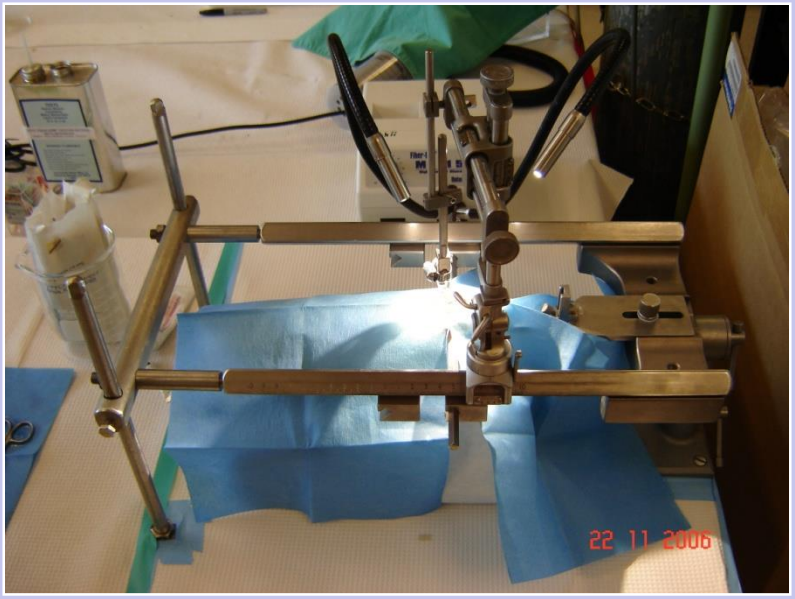
- Insulin and/or IGF signaling (Path B) normally maintains adult brain mass.
- Predicts that insulin and/or IGF prevents the loss of brain mass via Path B independently of Path A hyperglycemia (discriminatory test).



- Tiny i.c.v. insulin and IGF doses with *no* effect on hyperglycemia.

STZ Rat Model of Type I Diabetes

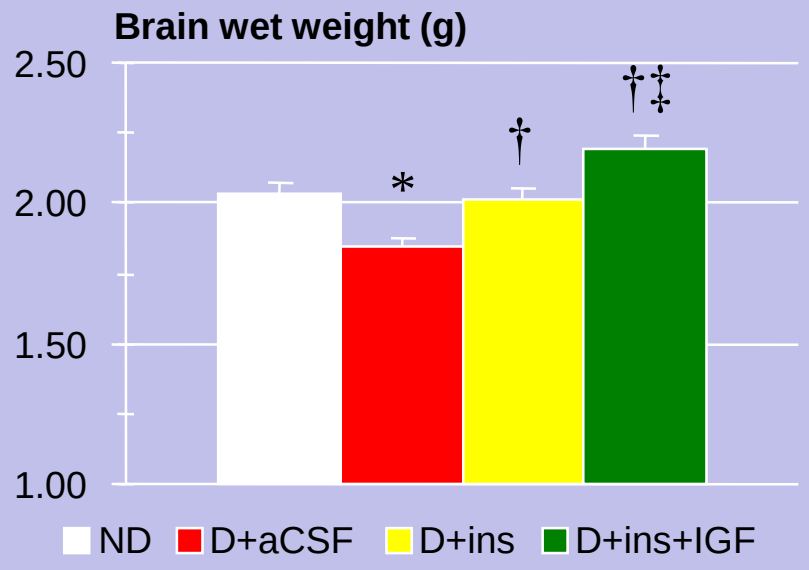
- Experiment:
  - **ND (n=8)**
  - **D+aCSF (n=9)**
  - **D+ins (n=9)**
  - **D+ins+IGF (n=9)**

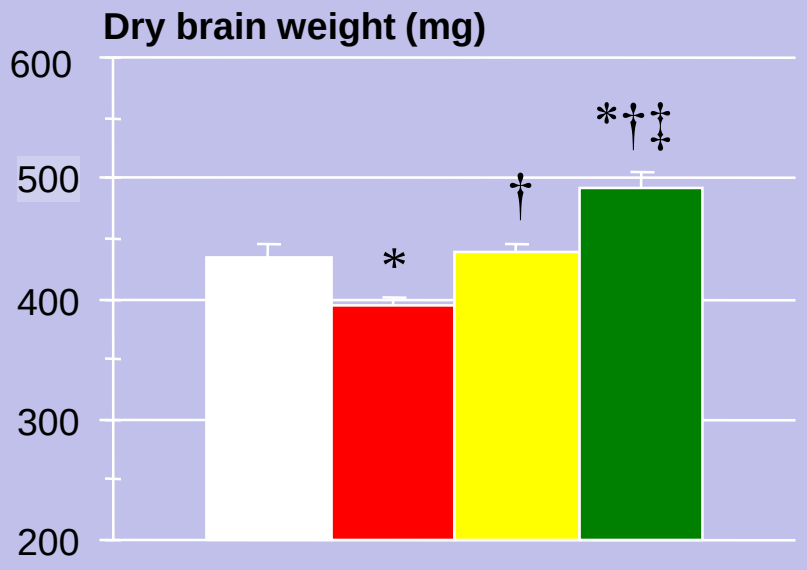


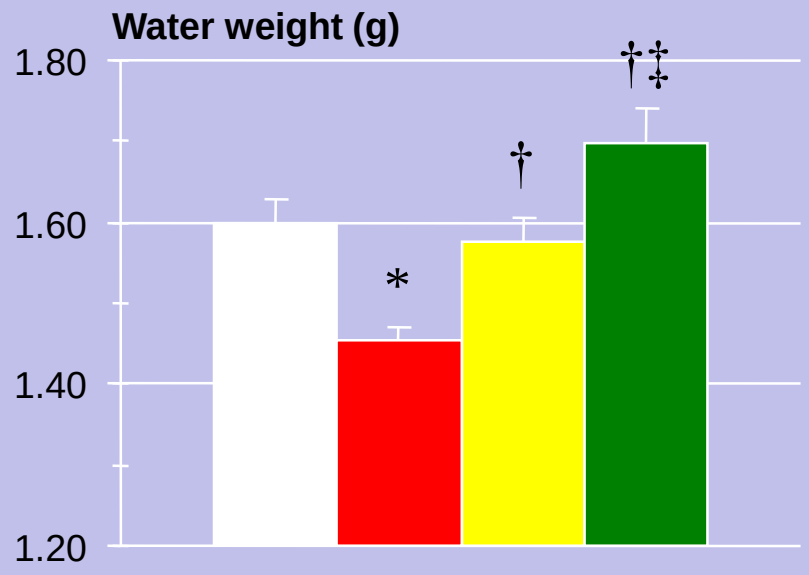
  - Doses (i.c.v.):
    - Insulin: 509 pmol/day.
    - IGF-I: 65 pmol/day.
  - Duration of diabetes: 12 weeks.
  - Some additional animals were fixed for IHC.

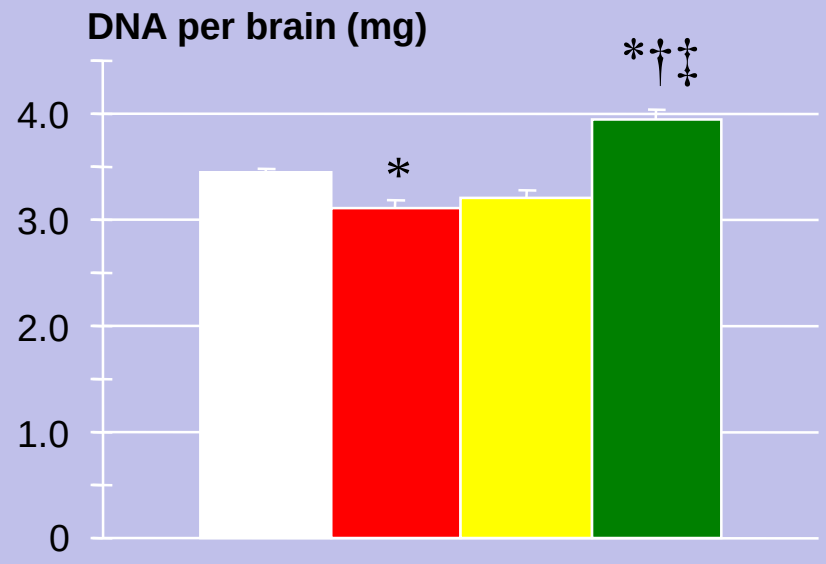
  - HYPOTHESIS: Brain atrophy can be prevented *despite* unabated hyperglycemia (discriminating test).

Insulin & IGF-I Preserve Brain Mass

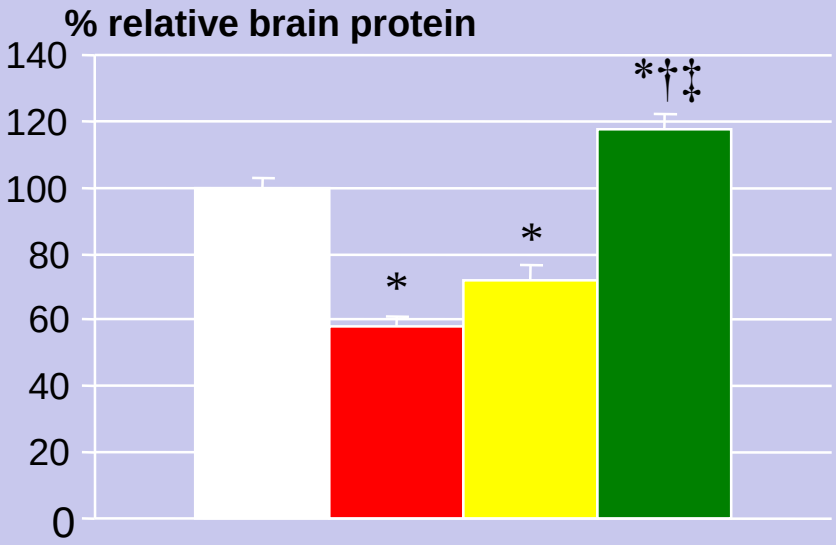


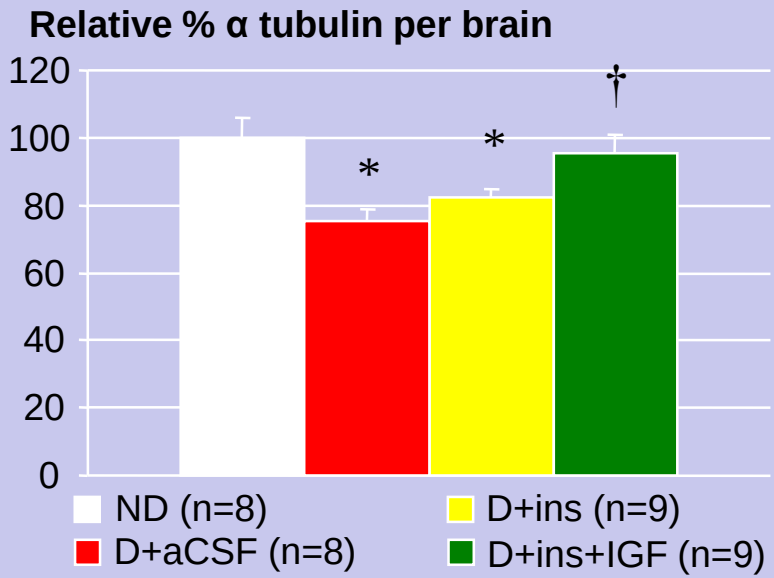


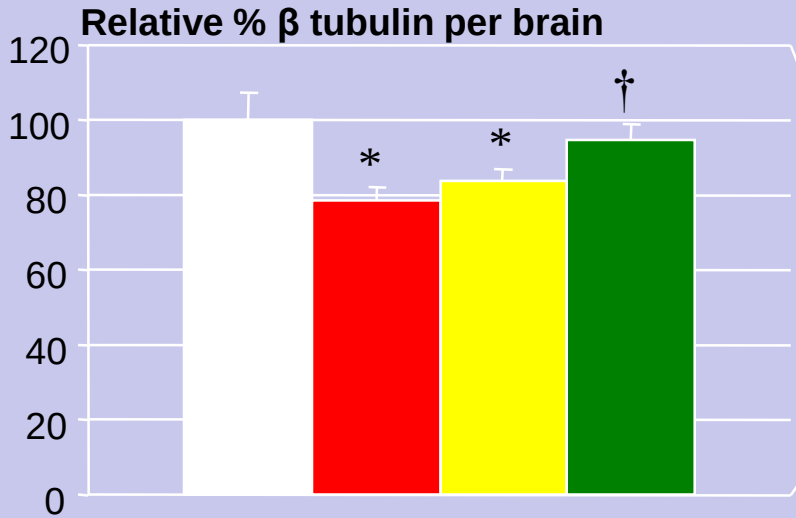


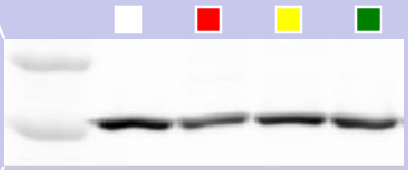


Insulin & IGF-I prevent loss of major brain proteins

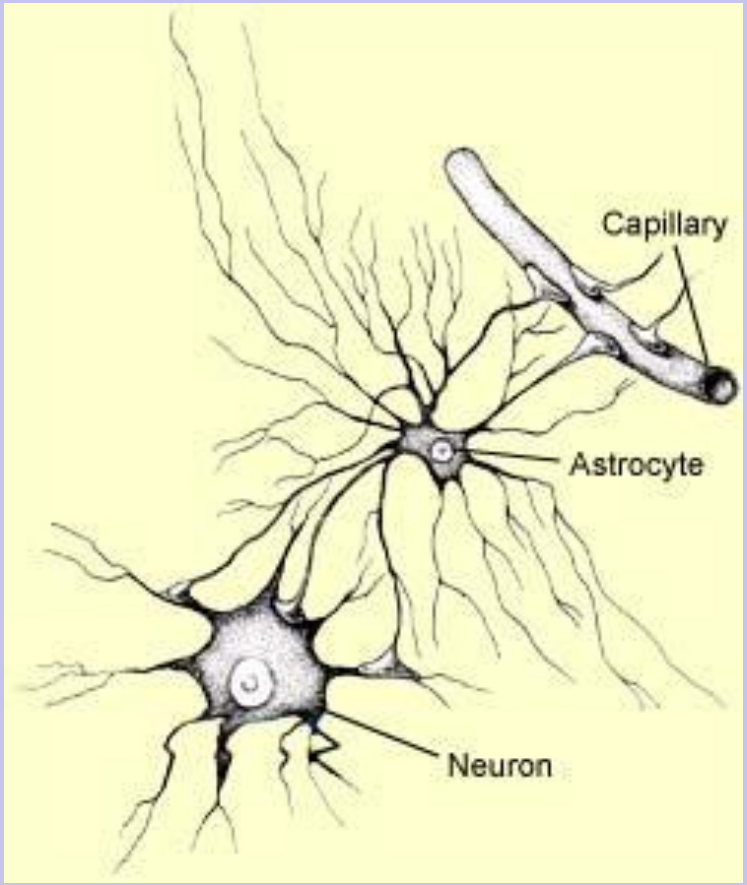


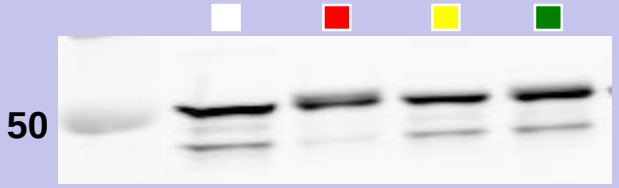


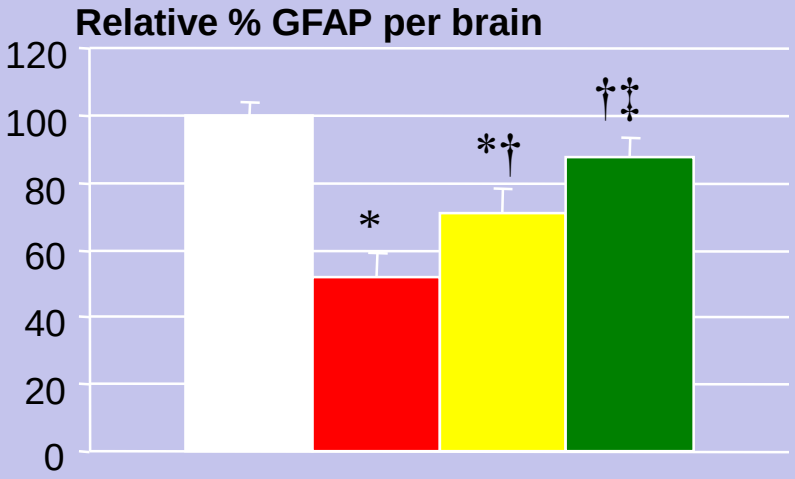


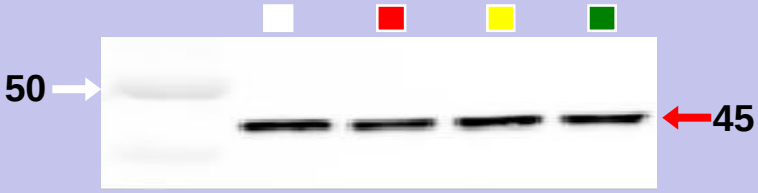


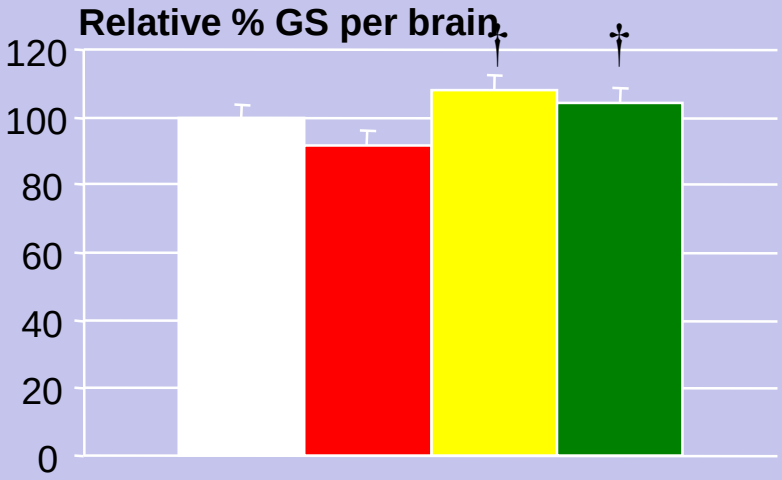
Astrocyte-specific proteins



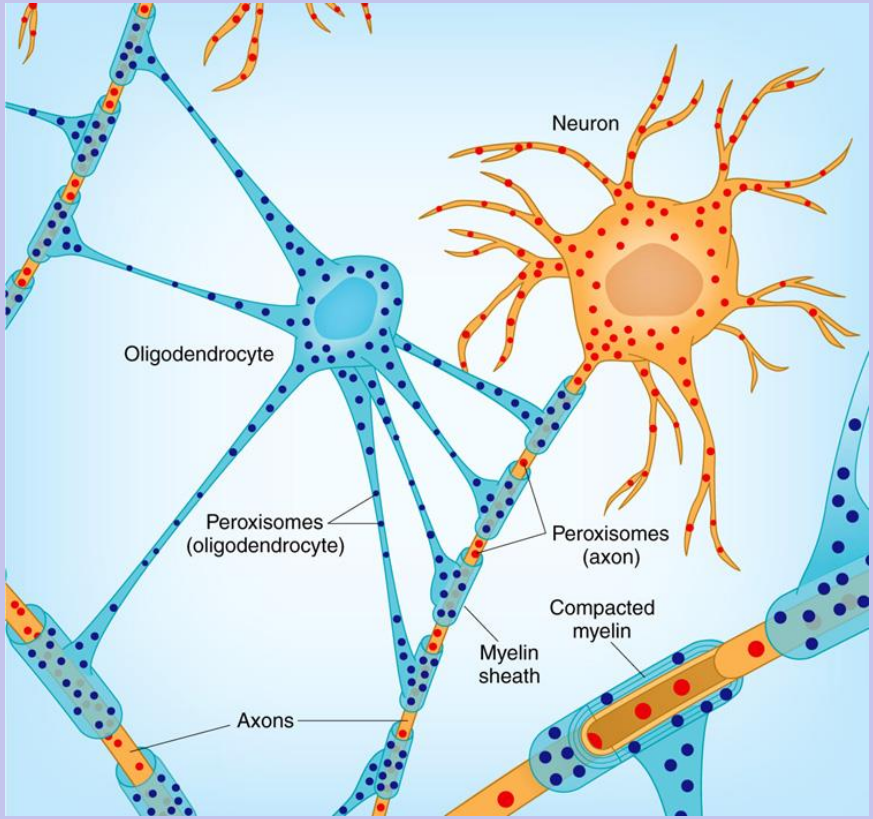


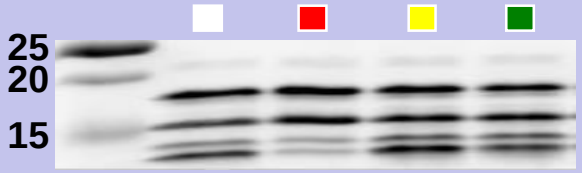


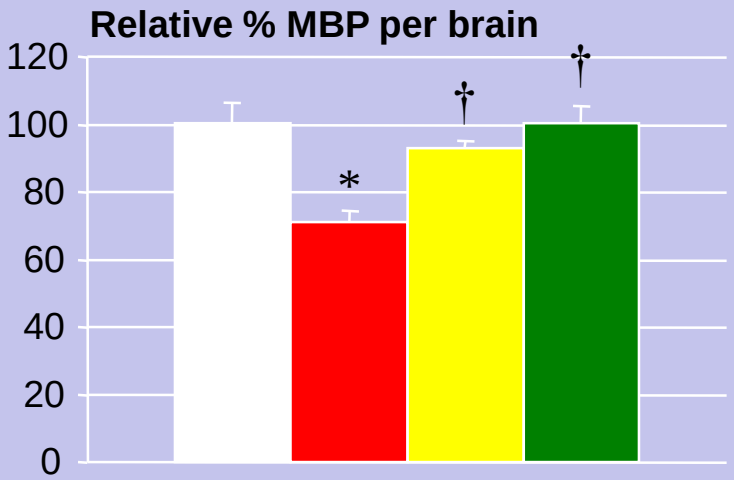


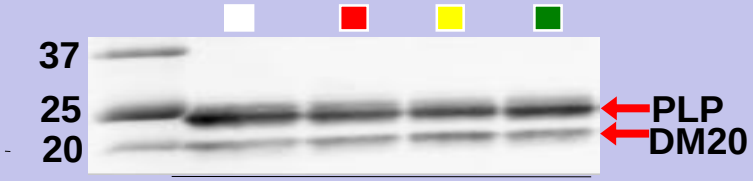


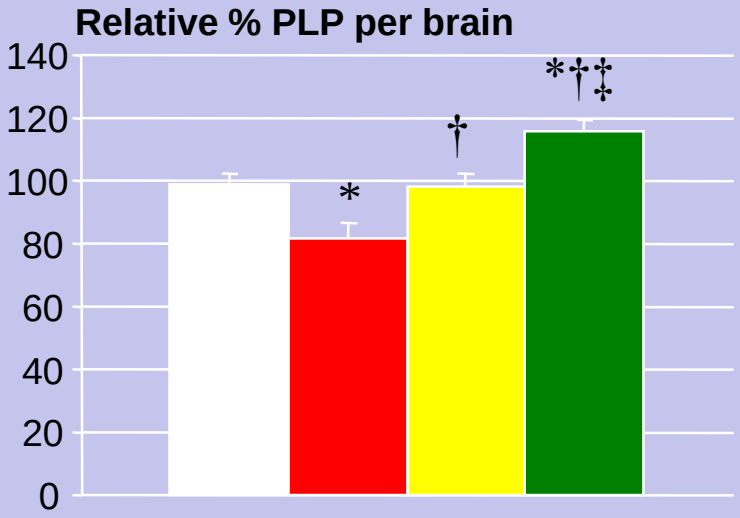
Oligodendrocyte-specific proteins



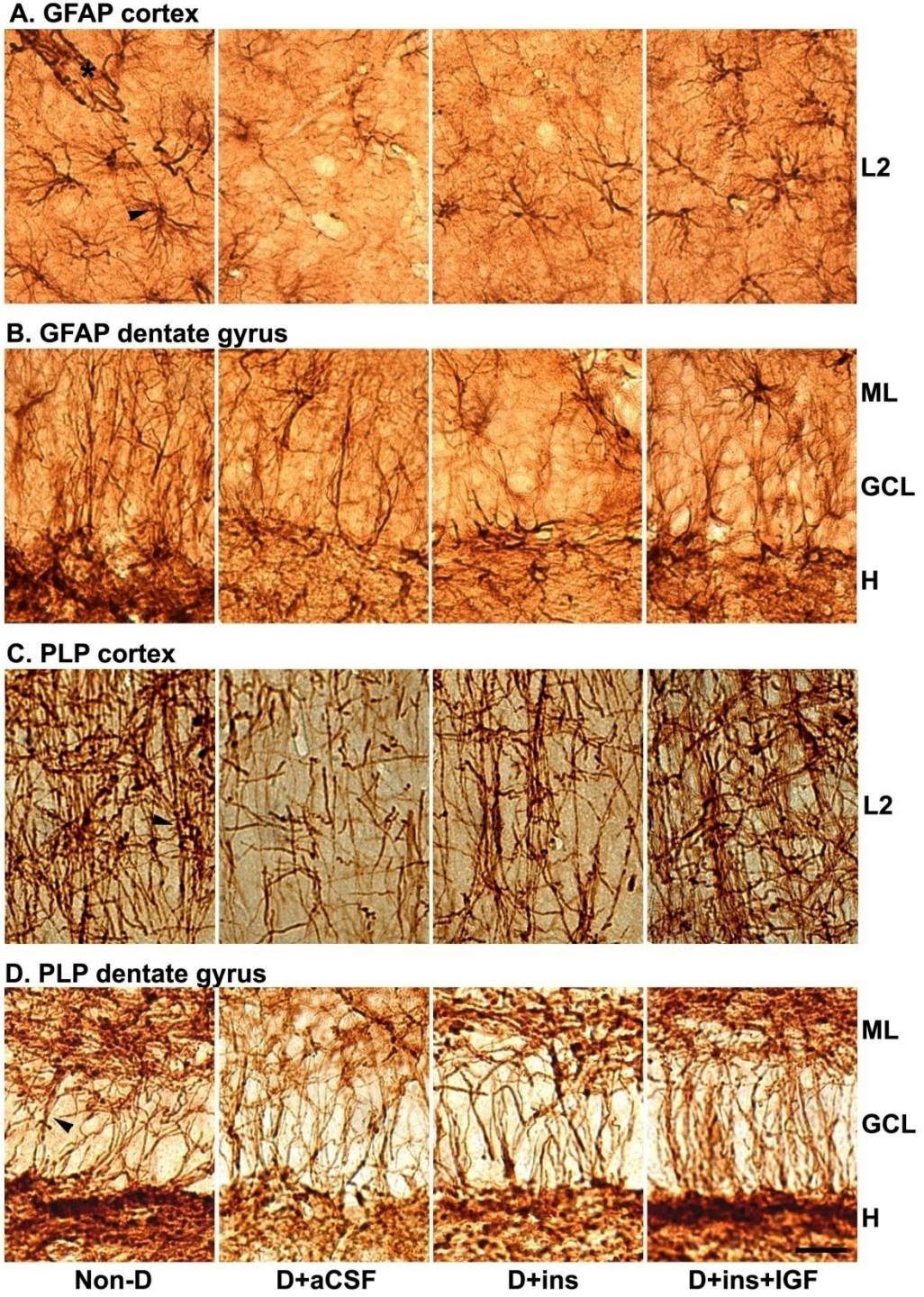




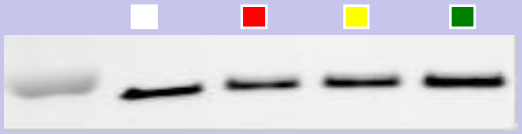


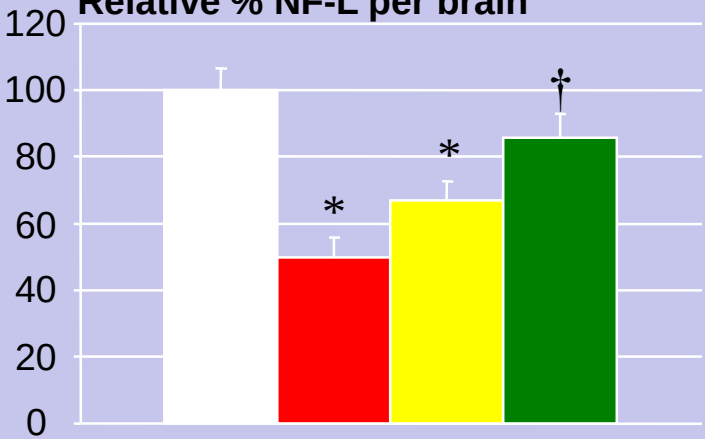


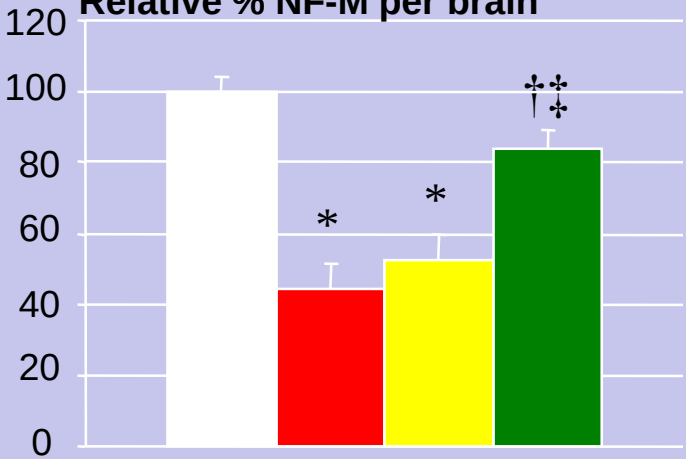
Insulin & IGF prevent the loss of glial-specific GFAP and PLP in brain slices from the cortex and hippocampal formation of diabetic rats.

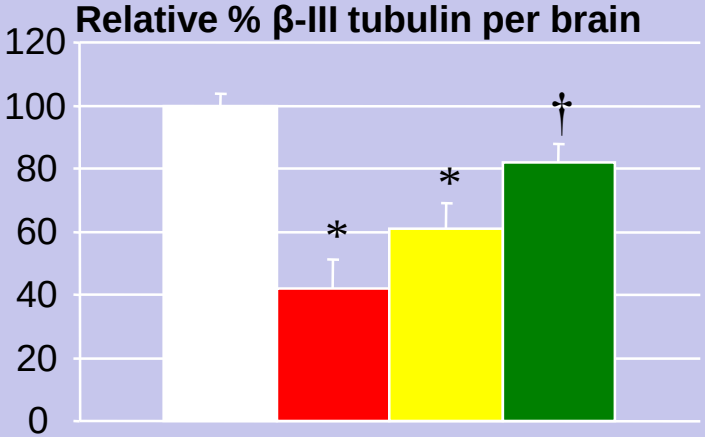


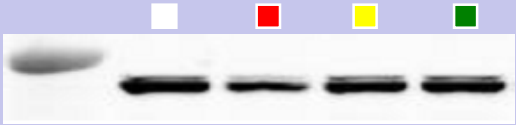
Neuron-specific proteins

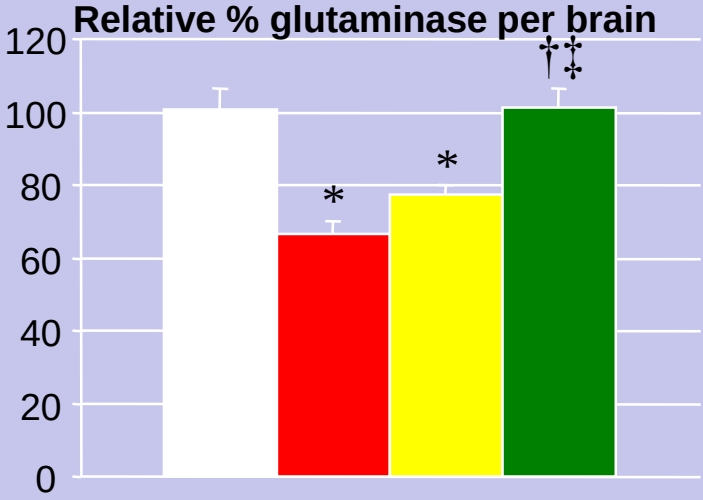




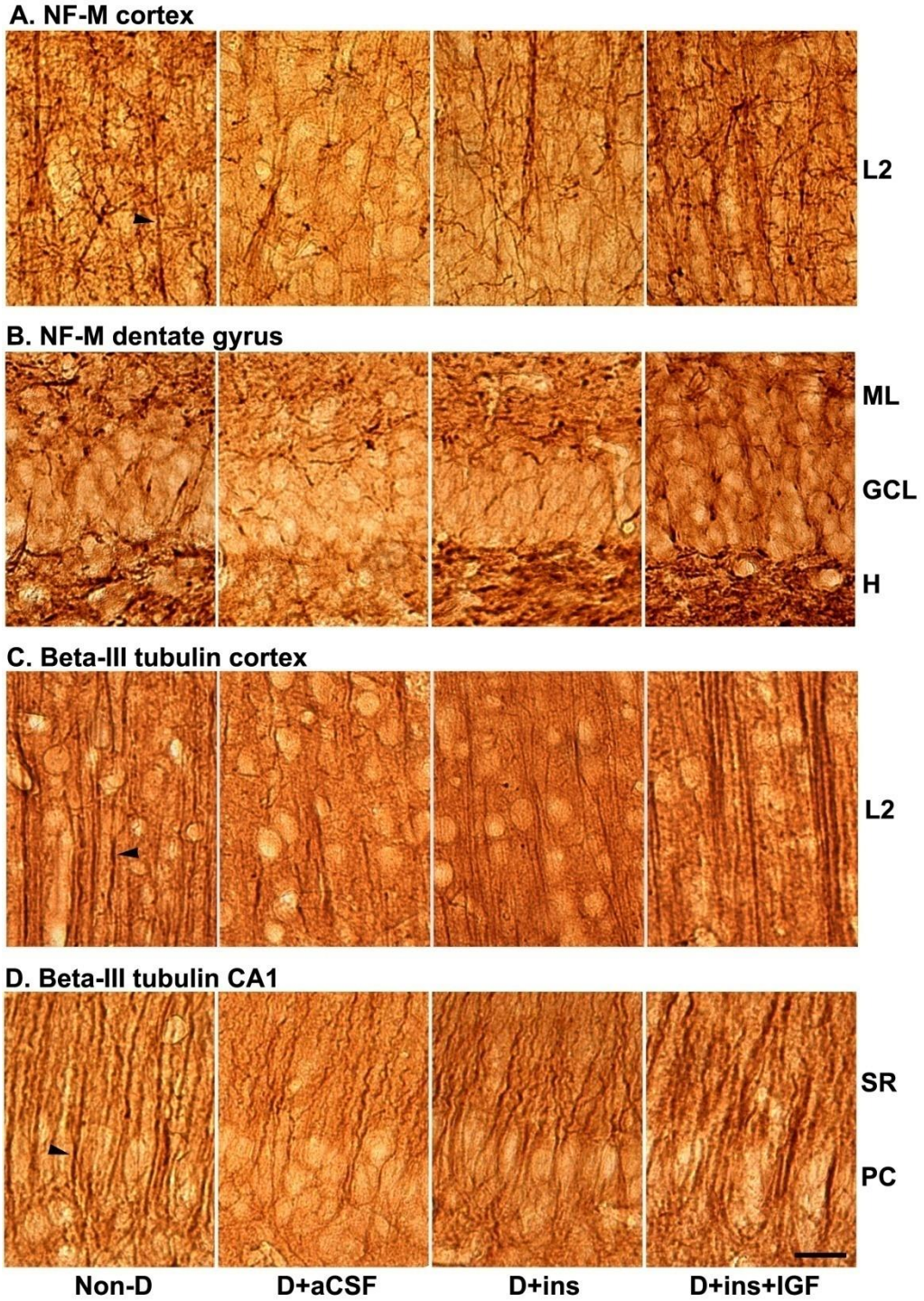




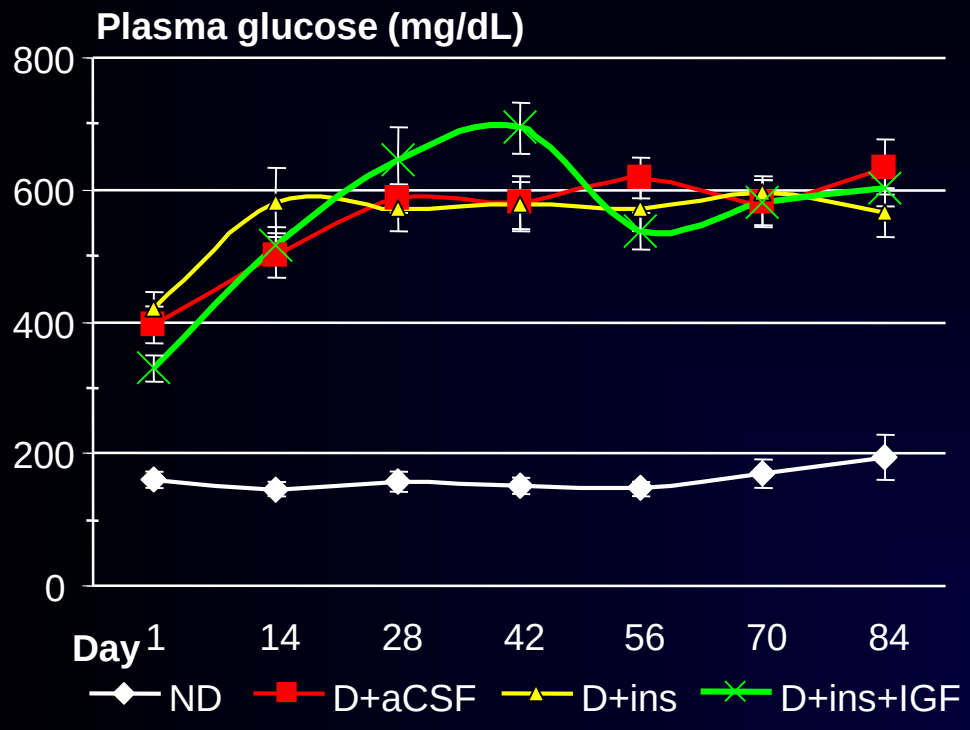


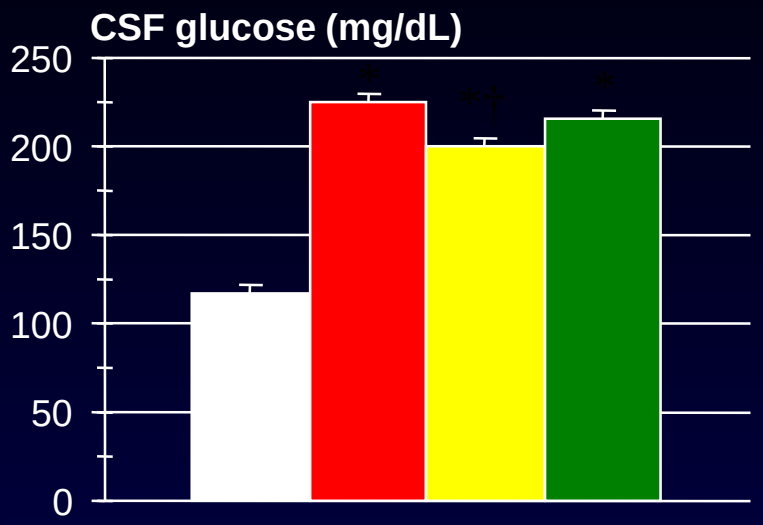


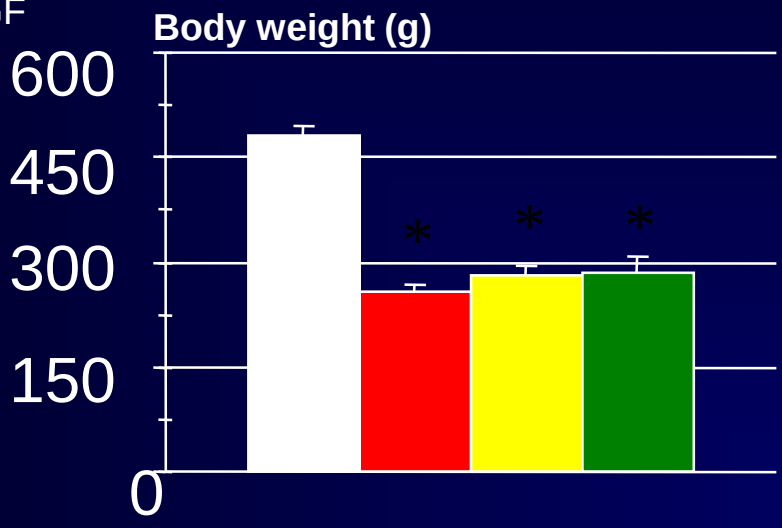
Insulin & IGF prevent loss of neuronal NF-M and Beta-III tubulin in brain slices from cortex and hippocampal formation in diabetic rats.



No Effect on Hyperglycemia Nor Weight Loss







Conclusions (From Serbedzija et al., 2009)

- First identification of brain proteins that control adult brain mass
- Loss of both insulin and IGF in diabetic rats caused loss of:
  - brain wet, water and dry mass.
  - total protein and DNA.
  - glial GFAP, PLP and MBP.
  - neuronal NF-M, NF-L and β-III tubulin
  - Note: NIRKO mice; IRS2 KO mice
- Insulin treatment:
  - prevented the loss of brain wet, water and dry mass.
  - did *not* prevent loss of body weight (independent regulation)
  - prevented the loss of glial proteins PLP, MBP, and GFAP.
  - was effective despite ongoing hyperglycemia, polyols, glycation, AGE, and their effects on microvasculature.
- Both insulin and IGF required to fully prevent brain atrophy

Implication for the Hyperglycemia Hypothesis

- **Many hundred of proteins are involved in the complex neurophysiological disturbances tested in the peripheral and central nervous systems.**
- **After 12 weeks diabetes, all susceptible proteins are undoubtedly maximally glycated (glucose 3-4X normal), yet such glycation does not prevent insulin/IGF from normalizing brain mass and actions on neurons, astrocytes and oligodendrocytes.**
- **Principal: Hyperglycemia (including increased polyols, protein glycation, AGE, etc.) is ASSOCIATED, but NOT the main CAUSE of diabetic neurological complications!**
- **Microangiopathy due to glycation and AGE is NOT the main cause of diabetic neurological complications.**
- **One must now reconsider whether hyperglycemia *per se* is the main cause of *other* diabetic complications.**

Implication for the Hyperglycemia Hypothesis (cont.)

- Clinical trials show that normalization of glucose can reduce the incidence of amputations in diabetes.
- Such amputations are secondary to infections in deep wounds that heal slowly and poorly.
- High glucose levels may exacerbate the progression of infections.
- Unknown whether IGF treatment may increase rate of nerve regeneration, hasten wound healing, and thereby reduce risk of amputations.

Diabetic Neurological Complications

- **Insulin and IGF prevent adult brain atrophy by retaining total RNA and protein levels in neurons & glia.**
- **Insulin resistance with diminished IGF activity, *not* hyperglycemia, is the predominant risk factor for brain atrophy and LOAD**
- **Intranasal insulin prevents cognitive decline in MCI and recent onset LOAD, with no effect on circulating glucose in a phase II clinical trial (Craft et al., 2011)**
- **Insulin and IGFs, most likely, similarly support the PNS**
- **Hundreds of publications may require reinterpretation.**
- **It remains important to prevent excessive hypergly-cemia: electrolyte loss, dehydration, acidosis, hyperosmolar conditions, etc.**

Concerning insulin use in clinical trials

- The alternative hypothesis proposes that insulin has two relatively independent effects on patients:
- It reduces glucose
- It also regulates protein levels in tissues (transcription, translation, protein modification)
- In a T1D clinical trial, systemic insulin treatment (s.c., i.m., i.v.) would alter both variables, hence one may not know which variable causes betterment in complications.
- The systemic effects on hyperglycemia can be avoided by i.c.v. insulin infusion (tiny doses) into the brain

Why anti-hyperglycemia therapy fails in clinical trials

- **Insulin treatment is partially effective against complications in T1D.**
  - **Insulin resistance is generally low**
  - **Insulin regulation of protein levels may be main reason for betterment, not reduction of hyperglycemia**
  - **Insulin therapy does not fully restore IGF levels**
  - **IGF supplementation may provide improved protection.**
- **Anti-hyperglycemia therapy fails in T2D**
  - **Insulin resistance blocks response**
  - **Oral agents that reduce hyperglycemia, are not known to increase protein levels, and exposes to adverse risk**

Many anomalies to HH now explained

- **Typical DPN is symmetrical, nerve length-dependent, sensorimotor polyneuropathy. Largest risk is long-standing hyperglycemia.**

  Why a progressive, long-standing disease: slow age-dependent loss of insulin/IGF (Tan & Baxter, 1986).

  Why same level of hyperglycemia, but lower risk in young *vs.* older patients: threshold & age-dependent loss of IGFs.

  Why is there nerve length dependency in risk (stocking-glove): longer nerves require greater protein synthesis, which is compromised.

  Why a separate time-course for DPN, autonomic and central dysfunction: Neurons are supported by insulin, IGF, and other neurotrophic factors. Various tissues have differing requirements and sensitivities to these factors.

Many anomalies to HH now explained (cont.)

- **Why is obesity a major risk factor: IGF already partially reduced & insulin resistance.**
- **Why exercise helps DPN: it increases IGF levels.**
- **Why insulin treatment neuritis: initial protein level imbalance**
- **Why at HbA1c < 5.4%, 40% develop DPN**
- **Birds have hyperglycemia and elevated body temperatures**
  - **Should have more extensive protein glycation**
  - **Why don't they have diabetic complications?**
- **Why primary kidney, retinal, neurons and glial cells can flourish in media with 4-fold elevated glucose concentration: its not the glucose.**
- **Why are microvessel abnormalities associated with DPN: Perhaps due to impaired protein synthesis, not hyperglycemia.**

Recommendations

- The *interpretation* of many hundreds of research publications should be re-examined: are results of treatment due to reduction of hyperglycemia, or increased tissue protein levels?
- Hyperglycemia should *not* be the main end-point in trials to prevent diabetic complications
- Highly questionable whether oral drugs should be used to control diabetic complications without adequate justification.
- FDA should reconsider hyperglycemia as main end-point for oral anti-diabetic drug approval
- Insulin resistance should be a primary target of treatment

Recommendations (cont.)

- Therapy should include IGF-1 (trial needed).
- IGF-1 should be manufactured and tested by nations
  - The IGF-1 molecule is in the public domain
  - Its manufacturing process is in the public domain
  - Its safety has been tested in children small for age
  - Because it may prove essential for treating large patient populations with diabetes or Alzheimer's, nations should act immediately to control its cost and availability.

Intranasal insulin should be manufactured and tested by nations.

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FINIS

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Dr. Ishii awarded 20 patents on use of insulin and IGFs to treat neurological disorders

Questions?

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