Experimentally Induced Diabetes in Transgenic AD Mice Accelerates Brain Pathology

Giulio Maria Pasinetti, M.D., Ph.D.
Mount Sinai School of Medicine
New York, NY
| • Rotterdam Study- Type II diabetes (non insulin dependant diabetes; NDDM) doubles RR (1.9) of AD incidence even when cases with cerebrovascular disorders were excluded (Ott et al., 1999). |
| • Insulin resistance, a major feature of Type II diabetes, is a significant risk factor pure AD (>2 fold RR). Association of diabetes and AD is strong among carriers of the ApoE4 (Peila 2002) |
| • Therapeutic evidence that certain insulin sensitizing drugs may beneficially influence AD: |
  - biguanide (e.g. metformin) |
  - glitazones (insulin sensitizing & PPAR-activating actions) |
The Potential Role of Diabetes in Alzheimer’s Disease

Lifestyle factors (high caloric intake)

Genetic Predisposition

Insulin Resistance

Aging

AD Dementia
- Amyloid ?
- NFT plaques ?
- Others?

Type II Diabetes
- Dislipidemia
- ↑Glycerides
- Hyperinsulinemia

VAD (Cardiovascular risk factors)
- Hypertension
- Atherosclerosis

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Diet Induced Diabetes in mouse models of Alzheimer’s Disease type Neuropathology

• Does insulin resistance promote AD type neuropathology through mechanisms that involve generation (e.g. γ-secretase) or impaired clearance (e.g. insulin degrading enzyme) of Aβ?

• The mechanism associated with insulin resistance mediated amyloidosis may involve abnormal regulation of insulin receptor (IR) functions in the brain.

• If insulin resistance promotes Aβ generation, are insulin sensitizing-anti-diabetic drugs beneficial to AD type amyloid neuropathology?
Potential Roles of Diet Induced Diabetes in Alzheimer’s Disease Neuropathology

Scheme of Treatment

Start High Calorie Diabetogenic Diet on Tg 2576

Ongoing
- Therapeutic efficacy of insulin sens.
- Brain pathology by non invasive MRI
- Relationship with cognition

• Aβ generation (secretase activities)
• Aβ clearance - IDE
• AD-type neuropathology
### Potential Roles of Diet-Induced Diabetes in Alzheimer’s Disease Neuropathology

#### Dietary composition

<table>
<thead>
<tr>
<th></th>
<th>High Fat</th>
<th>CTL</th>
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<tbody>
<tr>
<td><strong>Fat</strong></td>
<td>60%</td>
<td>20%</td>
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<tr>
<td><strong>Carbohydrate</strong></td>
<td>20%</td>
<td>60%</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td>20%</td>
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#### Effects of diabetogenic high fat diet
- ↑ obesity
- ↑ fat pat deposition
- ↑ triglyceride
- ↑ insulin
- ↑ hyperglycemia
- = serum cholesterol content
- ↑ insulin resistance – Gluc Toler test

- **Standard diet**
- **HF diet**

**Tg2576 mice**
Diet Induced Diabetes Promotes $\beta$-Peptide Content in the Brain

$\beta$-peptide (5M Guanidine extractable)

- **Control Group**
- **Insulin Resistant Group**

$\beta_{1-40}$

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<tr>
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<th>Hippocampal formation</th>
<th>Cerebral Cortex</th>
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<tr>
<td>6 Mo</td>
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<tr>
<td>8 Mo</td>
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$\beta_{1-42}$

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<td><img src="image7" alt="" /></td>
<td><img src="image8" alt="" /></td>
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</table>

- Means ± SEM, n= 3-4 per group; *P <0.01 vs control group
- Diet induced insulin resistance lasted for 3-5 months respectively starting at 3 month of age
Diet Induced Diabetes Promotes AD-type β-amyloid Plaque Neuropathology in the Tg2576 Mouse Brain

Stereological assessments of amyloid burden

Amyloid burden (% of cerebral cortex volume x 10^-4)

Tg Control
Tg Diet Induced Diabetes

N=4-6, P=0.01 ANOVA

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Potential Mechanisms Through which Diet Induced Diabetes May Influence AD β-amyloidosis in the Brain

1. β-secretase
2. γ-secretase

APP Mutations → Synthetic Pathway

IDE
Neprilysin

β-amyloidosis → Fibrillization

ApoEε4

Non-amyloidogenic pathway

Uptake/Clearance
Quantification of C-Terminal Fragment (CTF)-γ of APP as Index of γ-Secretase Activity

This in vitro γ-secretase assay allows APP/CTF-γ to be cleaved while membrane bound.

In Vitro γ-Secretase Assay based on detection of the putative C-terminal fragment of Aβ-PP (P6) (Sastre & Haass, 2001)

Isolate membrane fraction (100,000 × g) Control

Incubate at 37°C for 2 h Un-reacted control (4 °C)

Electrophoretic detection of C8 immunoreactive γ–CTF

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Diet Induced Diabetes Coincides with Increased $\gamma$-Secretase Activity in the Brain

Factors affecting A$\beta$ production

1. $\beta$-secretase
2. $\gamma$-secretase

Diet:           Control   Diabetes         Control  Diabetes

No Incubation Control

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<thead>
<tr>
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<th>Control</th>
<th>Diabetes</th>
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<tr>
<td>Holo-APP</td>
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<tr>
<td>$\alpha\beta$- CTF</td>
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<tr>
<td>$\gamma$-CTF</td>
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$\gamma$-CTF / holo-APP

Diet: Control Ins. Res.

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Means ± SEM, n= 3 per group; *P <0.01 vs control group

$\bullet$ Diet induced insulin resistance lasted for 5 months starting at 3 month of age

Diet: Control Ins. Res.

holo-APP

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Major Cleavage Sites for Metallopeptidases in the holo-APP Protein May Predict Degradation

Full length APP

NH₂ Full length APP COOH

β-secretase

γ-secretase (producing Aβ 1-40 or Aβ 1-42)

N A N I I E E N,E I N N M
KM / DAE / FRHD / SG / YEVH / H / QK / L / V / F / F / AEDVGSNK / GA / IIG / L / MVG / GVV / IA / TVI

A = angiotensin-converting enzyme
E = ECE-1
M = MNP-9
N = NEP
I = IDE

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Role of Insulin Degrading Enzyme in AD

- Aβ peptide levels in brain are inversely correlated with IDE and IDE influence γ–CTF degradation (Miller et al., 2003).

- IDE regulates the elevation of insulin, and its hypofunction (IDE KO) promotes Aβ generation in vivo (Farris et al., 2003).

- Reduced hippocampal IDE in late onset AD associated most strongly with APOE4 allelic content (Cook et al., 2003).
Diet induced diabetes in Tg2576 Mice Coincides with Decreased IDE Expression and Activity in the Brain

Factors affecting \( \alpha \beta \) production

1. IDE
2. Neprilysin

Degradation → Amyloidosis

IDE

-110KDa
-105KDa

APP

\( \alpha \beta \)

• Means ± SEM, n= 3 per group; *P <0.01 vs control group
• Diet induced insulin resistance lasted for 5 months starting at 3 month of age

Graph A:
- IDE Content (% of Control diet)
- Control Group vs Insulin Resistant Group

Graph B:
- Undegraded Insulin (% CPM)
- Degraded Insulin (% CPM)
- Insulin Resistant Group vs Control Group

Insulin resistance results in increased levels of undegraded insulin (\( \alpha \beta \))

...while resulting in decreased degraded insulin (\( \alpha \beta \))
Diet Induced Diabetes Coincides with Spatial Memory Impairment in a Water Maze Behavior Test

(8 months old Tg 2576 mice following 5 month diet leading to insulin resistance)

Escape latencies for **APP mice improved during the learning phase of water maze testing** (consistent at this age) while **Insulin Resistant APP mice maintained longer latencies** (suggesting that insulin resistance coincided with memory impairment).

APP mice showed a preference for the former platform location during the spatial probe test. While Insulin Resistant APP mice swam randomly across the 4 quadrants, suggesting impaired spatial learning.
The Role of Diet Induced Diabetes in Alzheimer’s Disease

What is the mechanism through which diabetes may promotes Aβ processing?
Diet Induced Diabetes Influences Insulin Receptor PY\textsuperscript{1162/1163-IR} in Absence of Detectable Change in Insulin Receptor Expression in the Cerebral Cortex

Implication in IR mediated signal transduction

- Insulin Resistance
- Changes in Signaling Pathway
- \(\beta\)-Amyloid Generation

8 month old Tg 2576 AD mice, 5 months of diet
Altered Insulin Receptor PY \textsubscript{1162/1163}-IR in Diabetic Tg 2576 Mice Coincides with Decreased MAP Kinase Phosphorylation and Increase GSK-\(\alpha\) and GSK-\(\beta\) Phosphorylation in the Cerebral Cortex.

8 month old Tg 2576 AD mice, 3 months of diet.
Activation of GSK-3 Correlates With Induction of $\gamma$-secretase activity in the brain of “diabetic” Tg2576 mice

A

$\gamma$-secretase activity

B

Correlation of $\gamma$-secretase activity with GSK-3 activities

Tg control

Tg insulin resistant

$\gamma$-secretase activity (as % of control)

Holo APP / Actin (Optical Density)

Phospho [S$^{21}$]GSK-3$\alpha$

Phospho [S$^9$]GSK-3$\beta$

“Active” GSK-3$\alpha$

“Active” GSK-3$\beta$
IRβ silencing decreases AKT and promotes GSK (decreased S9 and S21 phosphorylation) consistent with the evidence in “diabetic” Tg2576 mice

Means ± SEM, n= 3 per group in 2 independent studies
*P <0.01 vs control group
Neuronal IR - KO in “NIRKO” mice recapitulates altered signalling in the brain observed in “diabetic” Tg2576 mice

- Hemizygous NIRKO, collaboration with Dr. Accili
- Did not detect for GSK3α
The Role of Diabetes in AD

Lifestyle factors (e.g. diet)

Lifestyle factors (e.g. diet)

Insulin Resistance Diabetes

IDE

γ-secretase

Aβ-generation

Neurological Controls/ MCI

Accelerating factor in AD amyloid neuropathology

AD Dementia

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