

# Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease<sup>1</sup>

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## SPECIFIC AIMS

Insulin resistance, a proximal cause of Type II diabetes (a non-insulin-dependent form of diabetes mellitus (NIDDM)), is associated with an increased relative risk for Alzheimer's disease (AD). To clarify how NIDDM may influence Alzheimer's disease (AD) neuropathology, we explore the role of experimental NIDDM-like insulin resistance in the Tg2576 mouse model, which develops AD type neuropathology.

## PRINCIPAL FINDINGS

1. We found that a diet that induces insulin resistance promoted amyloidogenic **β-amyloid** (Aβ) Aβ<sub>1-40</sub> and Aβ<sub>1-42</sub> peptide generation (Fig. 1A) and amyloid plaque burden (Fig. 1B) in the brain of Tg2576 mice that corresponded with increased γ-secretase activities and decreased insulin-degrading enzyme (IDE) activity. Moreover, increased Aβ production coincided with increased AD-type amyloid plaque burden in the brain and impaired performance in a spatial water maze task (see online article).

2. Further exploration of the apparent interrelationship of insulin resistance to brain amyloidosis revealed a functional decrease in insulin receptor (IR)-mediated signal transduction in the brain, as suggested by decreased IR β-subunit (IRβ) Y<sup>1162/1163</sup> autophosphorylation and reduced phosphatidylinositol 3 (PI3)-kinase/pS<sup>473</sup>-AKT/Protein kinase (PK)-B in these same brain regions.

3. In view of the inhibitory role of AKT/ PKB on glycogen synthase kinase (GSK)-3α activity that has been implicated in mechanisms associated with promotion of Aβ peptide generation, we explored the potential inter-relationship between GSK-3 and γ-secretase activity. We found that decreased pS<sup>21</sup>-GSK-3α and pS<sup>9</sup>-GSK-3β phosphorylation (indicative of GSK-3α/β activation) positively correlated with γ-secretase activity in the brain of insulin-resistant relative vs. normoglycemic Tg2576 mice (Fig. 2A–C).

## CONCLUSIONS AND SIGNIFICANCE

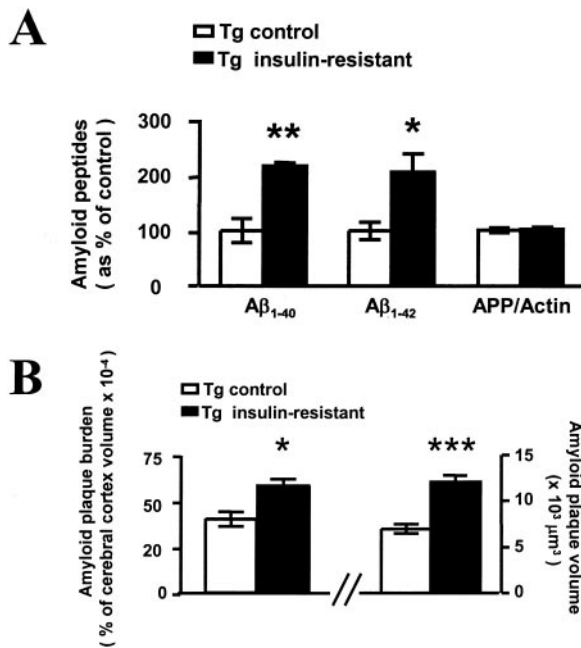
While AD accounts for most age-related dementia, AD is clinically and neuropathologically similar to other forms of dementia, e.g., vascular dementia (VaD). Further complicating interpretation of dementia type, a large body of recent evidence suggests that cardiovascular risk factors (CvRFs) (diet, life style factors, etc.) may promote pure AD-related neuropathology and AD dementia even when VaD cases are excluded from the analysis. Thus, it may be that additional nonvascular pathogenic events are related to the increased risk of AD conferred by certain CvRFs (e.g., insulin resistance). Using the Tg2576 mouse model of AD-type neuropathology, we explored a potential cause-effect relationship between dietary conditions described previously to promote NIDDM-like insulin resistance and AD neuropathology. We report two major observations supporting the hypothesis that CvRFs such as insulin resistance may directly promote the risk of AD-related neuropathology.

Our first observation is that diet-induced NIDDM-like insulin resistance results in the promotion of AD-type amyloidosis in the brain coincidental to altered IR signaling in the brain. We found that NIDDM-like insulin resistance in Tg2576 mice significantly attenuated IR signaling in the brain as assessed by reduced levels of Y<sup>1162/1163</sup>-IRβ phosphorylation whereas the total content of IRβ remained unchanged. This evidence is of high interest in that it supports the hypothesis that NIDDM-like insulin resistance in Tg2576 mice might influence signal transduction mechanisms downstream of the IR, which are known to promote amyloidogenic Aβ peptide generation as shown for GSK-3. Moreover, we found that increased activation of GSK-3α and GSK-3β (as reflected by decreased pS<sup>21</sup>-GSK-3α and pS<sup>9</sup>-GSK-3β) strongly correlated with increased γ-secretase activity in the brain of insulin-

<sup>1</sup> To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.03-0978fje>; doi: 10.1096/fj.03-0978fje

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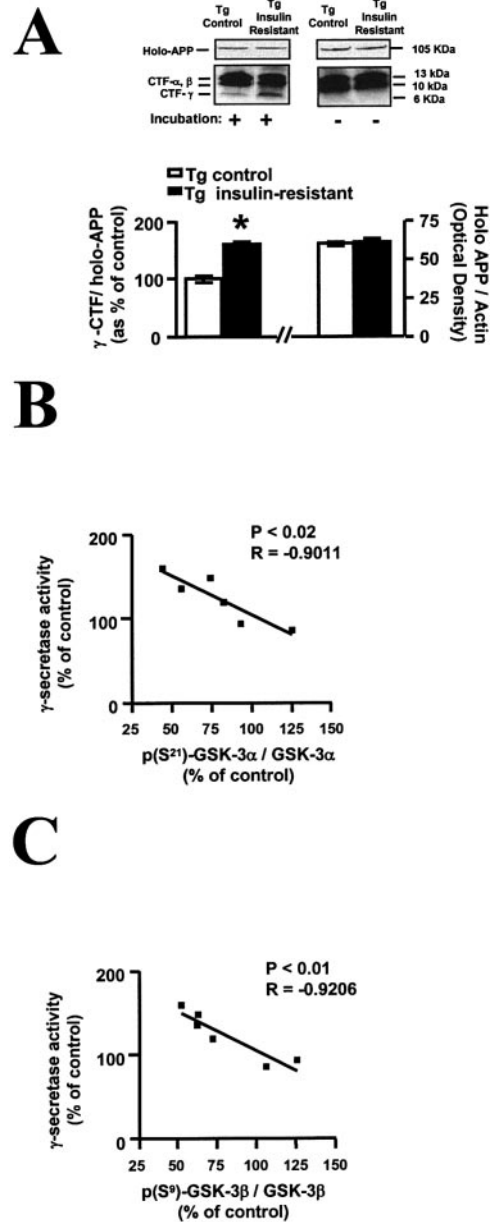
**Figure 1.** Diet-induced insulin resistance-mediated promotion of AD-type amyloid burden is associated with decreased IR signaling in the brain of Tg2576 mice. In this study nine-month-old insulin-resistant and normoglycemic Tg2576 female mice were assessed for indexes of AD-type amyloid burden. *A*) ELISA detection of Aβ<sub>1-40</sub> and Aβ<sub>1-42</sub> peptide content and 22C11 immunopositive holo-amyloid precursor protein (APP) content in the hippocampal formation. *B*) Stereological cerebral cortical (neocortex and hippocampal formation) 6E10 immunopositive amyloid plaque volume and burden (amyloid plaque volume as a % of cerebral cortex volume). *A, B*) values represent means ± SE, *n* = 5 or 6 per group; \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 vs. control group (2-tailed Student's *t* test).

resistant Tg2576 mice. This evidence suggests that in Tg2576 mice, dietary conditions leading to NIDDM-like insulin resistance may result in altered IR signaling in the brain and eventually “unleash” GSK-3α (and GSK-3β) activity implicated in the promotion of γ-secretase activity and the generation of amyloidogenic Aβ peptides.

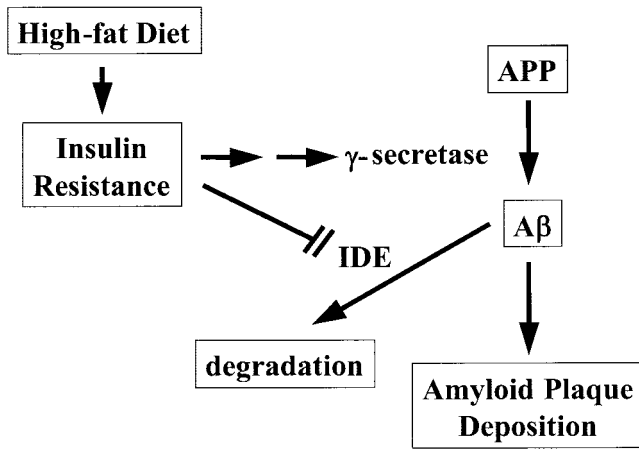
Our second observation was a significant decrease of IDE expression and activity in the brain of insulin-resistant Tg2576 mice, suggesting that NIDDM-like insulin resistance may have contributed to amyloidosis by interfering with IDE-mediated degradation of amyloidogenic Aβ peptides. A role for IDE in Aβ degradation was demonstrated by recent studies showing that mice deficient for IDE exhibit increased cerebral accumulation of endogenous Aβ peptides, as well as hyperinsulinemia and glucose intolerance, both hallmarks of NIDDM. Therefore, it would not be unexpected that hyperinsulinemia in response to NIDDM (as we found out in a diet-induced NIDDM mouse model) could further impede Aβ degradation via competition with insulin for degradation by IDE.

Our study presents the first experimental evidence to suggest that dietary treatment leading to a condition that recapitulates NIDDM in humans (e.g., hyperinsu-

linemia, glucose intolerance, and increased body weight) can promote AD-type amyloidosis in the brain independent of altered cholesterol metabolism. This important given earlier evidence indicating that exposure of Tg2576 mice to a diet rich in cholesterol, can result in elevated cholesterol content in brain (and



**Figure 2.** Insulin resistance promotes γ-secretase activity in the brain of Tg2576 mice. *A*) Increased γ-secretase activity in cerebral cortex (left panel) assessed by generation of γ-C-terminal fragment (CTF) from membrane associated APP (normalized to holo-APP in the membrane preparation). Parallel Western blot analysis of holo-APP from total brain extract (right panel) confirmed that the diet-induced insulin resistance did not influence holo-APP expression. Inset: Representative cleavage products generated from membrane-bound APP; as expected, negative controls (no incubation) yielded no CTF-γ cleavage product. *B, C*) Scatter plot analysis of γ-secretase activity as a function of pS<sup>21</sup>-GSK-3α/GSK-3α (*B*) and pS<sup>9</sup>-GSK-3β/GSK-3β (*C*). Straight line represents best linear regression fit.



**Figure 3.** Diet-induced insulin resistance may promote AD-type amyloidosis via mechanisms involving  $\gamma$ -secretase and insulin-degrading enzyme (IDE).

serum) and a subsequent promotion of brain amyloid deposition. For this reason we designed the present study to preclude this potential confound by using diets containing  $\sim 100$  fold less cholesterol than that of previous cholesterol-based studies. Moreover, in control studies, we confirmed that diet-induced NIDDM-like insulin resistance Tg2576 mice in our study maintained normal levels of cholesterol in serum and the brain. Thus, our study specifically demonstrates a novel link between impaired IR signaling under NIDDM-like insulin resistance conditions and the promotion of amyloidosis and cognitive impairment, independent of changes in cholesterol content in the periphery and in the brain.

In a series of control studies, we explored the glyce-mic status of Tg2576 mice compared with WT animals so that the potential influence of A $\beta$  itself on normal insulin signaling could be investigated. We found no intrinsic alteration in baseline glucose utilization rela-

tive to strain/age/gender-matched WT control mice as assessed by a glucose tolerance test. This finding suggests that the content of amyloidogenic A $\beta$  peptides in the brain of normoglycemic Tg2576 mice does not predicate conditions of altered glucose metabolism and that it is likely that the physiologic conditions resultant to NIDDM-like insulin resistance were responsible for the observed promotion of amyloidosis in Tg 2576 mice in this study. However, we note that previous evidence has indicated that Tg2576 mice have impaired plasma glucocorticoid and glucose regulation in response to restraint stress.

While it is well established that CvRFs are significant and potent risks factor for AD, our study for the first time provides evidence supporting a potential direct role of diet induced NIDDM-like insulin resistance conditions on AD neuropathology via the promotion of amyloidogenesis. Our studies strongly suggest that one mechanism by which diet-induced insulin resistance in Tg2576 mice can significantly promote AD-type amyloidosis in the brain is through impairment of IR signaling resulting in elevation of  $\gamma$ -secretase activities. Our studies also suggest that NIDDM may further contribute to amyloidosis by attenuating A $\beta$  degradation by mechanisms related to IDE.

In light of the growing incidence of NIDDM (currently >15 million cases in the U.S. alone) and the pervasiveness of insulin abnormalities in AD, even a modest therapeutic benefit gained by “insulin sensitization” would bear immeasurable benefit to public health. We suggest that the present model of diet-induced insulin resistance in Tg2576 AD mice is a simple, physiologically relevant methodology for studying the role of insulin resistance in AD neuropathology and may provide an efficient means for further evaluating the multiple insulin-sensitizer drugs and possibly GSK-3 inhibitors under consideration in AD clinical trials. FJ