Published in final edited form as:

Behav Brain Res. 2011 January 1; 216(1): 255–261. doi:10.1016/j.bbr.2010.08.002.

Rosiglitazone Reversal of Tg2576 Cognitive Deficits is Independent of Peripheral Gluco-Regulatory Status

Jennifer Rodriguez-Rivera^{a,b,f}, Larry Denner^{c,d,e,f}, and Kelly T. Dineley^{a,f,*}

^aDepartment of Neurology, University of Texas Medical Branch, Galveston, TX, United States, 77555

^bDepartment of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX, United States, 77555

^cNelda C. and H.J. Lutchter Stark Diabetes Center, University of Texas Medical Branch, Galveston, TX, United States, 77555

^dMiriam and Emmett McCoy Stem Cells and Diabetes Mass Spectrometry Research Laboratory, University of Texas Medical Branch Galveston, TX, United States, 77555

^eDepartment of Internal Medicine, Division of Endocrinology, University of Texas Medical Branch Galveston, TX, United States, 77555

^fMitchell Center for Neurodegenerative Diseases, University of Texas Medical Branch Galveston, TX, United States, 77555

Abstract

Converging lines of evidence associate gluco-regulatory abnormalities and peroxisome-proliferator-activated receptor (PPAR) gamma function with increased risk for Alzheimer's disease (AD). In this study, we used the Tg2576 AD mouse model to test the hypothesis that cognitive improvement following one-month of PPAR gamma agonism with rosiglitazone (RTZ) correlates with peripheral gluco-regulatory status. We assessed cognition and peripheral gluco-regulatory status of Tg2576 mice following one-month treatment with RTZ initiated prior to, coincident with, or after, the onset of peripheral gluco-regulatory abnormalities (4, 8, and 12-months of age, respectively). Whereas 5-months-old (MO) and 13 MO Tg2576 did not gain cognitive improvement after one-month treatment with RTZ, 9 MO Tg2576 mice exhibited reversal of associative learning and memory deficits. Peripheral gluco-regulatory abnormalities were improved in 9 and 13 MO Tg2576 with RTZ treatment; RTZ treatment had no effect on the normal glucose status of 5 MO Tg2576 mice. These findings suggest that RTZ-mediated cognitive improvement does not correlate with peripheral gluco-regulatory abnormalities per se, but reflects the age-dependent mechanistic differences that underlie cognitive decline in this mouse model.

© 2010 Elsevier B.V. All rights reserved.

*Corresponding author. Tel.: (409) 747-7060; Fax: (409) 747-7050, Mailing Address: 301 University Blvd, Galveston, TX 77555-0616, ktdinele@utmb.edu (K.T. Dineley).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

PPAR γ ; fear conditioning; hippocampus-dependent learning and memory; mouse model; Alzheimer's disease; glucose tolerance test; insulin

---

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that constitutes 60 to 80% of all dementia cases [1]. It is estimated that 5.3 million Americans are currently suffering from the disease. Without advances in treatment, it is expected that the number of AD patients will double by the year 2050, obviating the need for new and effective therapies.

AD is marked by a decline in cognitive abilities, particularly in the acquisition and retrieval of new memories [2,3]. In recent years, epidemiological studies suggest that there is a link between peripheral gluco-regulatory abnormalities and AD [4–7]. For example, patients who suffer from severe peripheral insulin resistance and hyperinsulinemia experienced in type 2 diabetes mellitus (T2DM), have an approximately 65% increased risk of developing AD [8]. Likewise, many AD patients exhibit mild to moderate peripheral insulin resistance, elevated peripheral insulin, and dysregulation of glucose metabolism [9]. As such, peripheral hyperinsulinemia and gluco-regulatory abnormalities are thought to increase the risk of AD, and patients with AD are more likely to suffer from peripheral gluco-regulatory abnormalities than healthy older adults. Given the converging evidence associating peripheral gluco-regulatory abnormalities and cognitive function in AD, insulin-sensitizing drugs have been proposed as a possible treatment for AD. One such drug is rosiglitazone (RTZ; marketed as Avandia®). RTZ is a member of the class of insulin-sensitizing drugs called thiazolidinediones (TZD). RTZ increases insulin sensitivity by functioning as a ligand to activate the nuclear receptor peroxisome proliferator-activated receptor γ (PPAR γ). PPAR γ agonism results in not only increased transcription of insulin responsive genes, PPAR γ activation has additional pleiotropic effects on many other signaling pathways; many of which are requisite for neuronal homeostasis and plasticity [10].

Indeed, some clinical trials have reported positive results that may presage the future value of PPAR γ agonists like RTZ in AD. For example, Dr. Craft's group has shown cognitive benefit of RTZ in amnesic MCI and mild-to-moderate AD patients lacking the APOE ϵ 4 allele [11, 12]. Furthermore, this group reported that another TZD pioglitazone, improved cognitive function in AD and MCI patients with mild insulin-resistance more effectively than niteglinide which is a compound that acts as an insulin secretagogue (Watson et al., 2007; Society for Neuroscience Abstract 525.4). These studies suggest that the TZDs may confer a positive benefit for certain subsets of MCI and AD patients. However, the strengths and limitations of TZD treatment are not completely discernible (or apparent) from human studies. It is not clear whether normalizing gluco-regulatory abnormalities, such as peripheral insulin resistance, is alone sufficient enough to improve cognitive performance. Therefore, utilizing an AD mouse model to study the effectiveness of RTZ treatment on cognitive function provides an opportunity to illuminate key variables for optimizing TZD treatment in AD patients.

The transgenic animal line Tg2576 is an extensively characterized mouse model for AD that expresses the 695 splice-variant of the amyloid precursor protein (APP) containing the familial AD 'Swedish' mutation KM670/671NL [13]. Tg2576 mice exhibit a subset of behavioral and pathological features of AD including age-dependent accumulation of beta-amyloid (A β) with subsequent learning and memory deficits that worsen in an age-dependent manner [13–21]. Our previous work has established that 3 months old (MO) Tg2576 are cognitively normal, 5 MO Tg2576 are mildly cognitively-impaired, and 9 MO Tg2576 are severely cognitively-

impaired; cognitive function continues to decline as these animals age [14,19–21]. Since these animals do not suffer significant loss of neurons or neurodegeneration, these are not the underlying mechanisms for their age-dependent cognitive decline. A more likely explanation is that cognitive decline in Tg2576 mice is due to age-dependent alterations in the intra- and inter-neuronal signaling mechanisms responsible for synaptic plasticity, learning and memory.

A correlation between metabolism and cognition has been shown in several AD mouse models, and previous work on Tg2576 suggests that signs of peripheral gluco-regulatory abnormalities are apparent by 8 MO [22–26]. However, it is not known if earlier existing peripheral gluco-regulatory abnormalities contribute to the onset of cognitive deficits in Tg2576 mice (by 5 months of age) or if reversing peripheral gluco-regulatory abnormalities is sufficient to improve cognitive performance at any age.

In this study, we tested the hypothesis that cognitive improvement following one-month of RTZ treatment correlates with peripheral gluco-regulatory status. Therefore, we conducted a cross-sectional study in which we treated 4, 8, and 12 MO Tg2576 mice (and wild-type (WT) littermates) for one month with RTZ, then tested associative learning and memory performance concomitant with an assessment of peripheral gluco-regulatory status. We found that while 5 MO Tg2576 mice are cognitively-impaired, they do not exhibit any signs of peripheral gluco-regulatory abnormalities, which indicates that peripheral gluco-regulatory status is not a precipitating factor in Tg2576 cognitive impairment. In addition, we discovered that treatment with RTZ in 9 and 13 MO Tg2576 mice effectively normalizes peripheral gluco-regulatory abnormalities, but only reverses cognitive deficits for 9 MO Tg2576 mice. These results suggest that RTZ-mediated cognitive improvement does not correlate with peripheral gluco-regulatory abnormalities per se but likely reflects age-dependent mechanistic differences that underlie cognitive decline in this mouse model. Since RTZ effectiveness at ameliorating cognitive deficits is greater when initiated prior to chronic peripheral gluco-regulatory abnormalities; RTZ's effectiveness for ameliorating cognitive impairment in Tg2576 AD mice appears to have a limited therapeutic window.

2. Materials and Methods

2.1. Animals and experimental diet

Transgenic mice and non-transgenic littermates were bred by mating Tg2576 males, from our colony, with C57Bl6/SJL (F1) females (Jackson Lab, Bar Harbor ME). Animal breeding was performed in the University of Texas Medical Branch (UTMB) Animal Research Care Facility maintained under USDA standards. All experimental protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1986) and IACUC approval.

The animal colony was maintained at an ambient temperature between 21–23°C and humidity (45–50%) on a 12-hr light-dark cycle (lights on 0700–1900 hr). Mice were housed ($n \leq 5$ /cage) with free-access to water and grain-based food (+/- RTZ supplementation). The RTZ diet consisted of rosiglitazone maleate (Avandia®, GlaxoSmithKline) pulverized into control grain-based food (Bio-Serv) at 30 mg/kg, a dose chosen based on previous studies [22]. Four, 8, or 12 MO mice were divided into 4 testing groups based on genotype (WT or Tg2576) and diet (RTZ or control): 1) Tg2576, and 2) WT mice fed RTZ diet; 3) Tg2576, and 4) WT mice fed control diet. Mice were fed RTZ or control feed for 1 month. Daily food consumption was recorded and each mouse ate an average of 6 grams of feed per day (data not shown), corresponding to a daily dose of 0.18 mg of RTZ per mouse. There was no significant difference in the amount of food consumed among the different groups (One-way ANOVA $F(3, 36) = 0.43$, $p = 0.73$; data not shown). All testing was conducted during the light cycle phase.

2.2. Fear Conditioning

Fear-conditioning training was performed at the beginning of the animals' light cycle. Twelve to 15 mice were trained in the fear conditioning chamber for a total of 7 min following our standard fear-conditioning protocol (Dineley et al., 2002). Briefly, mice were placed in the training chamber and allowed to roam freely for 3 min after which they were exposed to a conditioned stimulus, a 30-sec acoustic white noise (80 dB) [27]. The CS was followed by an unconditioned stimulus (US), a 2-sec foot shock (0.8 mA). The CS-US pairing was repeated at the 5-min mark. Contextual fear learning was determined the following morning (~24hrs after training), by placing each mouse in the training context and recording the freezing behavior for 5 min. Following contextual testing, cued-fear learning was determined ~30 hrs after training by placing the animals in a different context (novel odor, lighting, cage floor, and visual cues). Baseline behavior was recorded for 3 min, after which the CS was presented for 3 min (data not shown). The Actimetrics Freeze Frame video capture software and analysis program was used [20]. Following behavioral testing, mice were perfused, with ice-cold PBS supplemented with protease and phosphatase inhibitors, and decapitated for whole-brain removal. Immediately following, the brain was dissected into various sub-regions and stored at -80°C until use.

2.3. Glucose Tolerance Test (GTT)

Baseline glucose measurements and GTT testing were performed at the beginning of the animals' light cycle. Sixteen hrs before behavioral testing, mice were fasted overnight with access to water *ad libitum*. Baseline glucose was obtained then each mouse received an intra-peritoneal injection of glucose (1.5 mg/kg). Blood glucose clearance was measured after injection at the following time points: 15, 30, 60, 90 and 120 min.

2.4. Fasted insulin measurement

Food, but not water, was removed from each mouse cage 16 hrs prior to behavioral testing. Tail blood samples (50–100 μL) were collected from fasted animals using a capillary tube. The blood samples were allowed to clot for 30 min, and then centrifuged to obtain the serum. Fasted insulin in serum was measured using Millipore's mouse endocrine multiplex assay according to manufacturer's instructions (Lincoplex). Briefly, serum samples (10 μL) were incubated overnight (16–18 hrs) at 4°C with anti-insulin antibody immobilized beads. Samples were washed then incubated with detection antibody cocktail for 60 min, then streptavidin-phycoerythrin for 30 min. After washing, the plate was read on a BioRad Bioplex. Values were determined by comparison to an insulin standard curve (range: 6.2 pM – 4500 pM).

2.5 Data Analysis

Area under the curve (AUC) analysis was determined using the trapezoidal method, and results were expressed as mean $\pm$ standard error of the mean (SEM). In this method, a fitted curve is described as a series of connected XY points. The area under each connecting segment forms a trapezoid. The AUC is the summation of the areas of each trapezoid. Statistical analysis for the AUC data was performed using one-tail Student's t-test.

Statistical analysis of biochemical and behavior data was accomplished with ANOVA, followed by *post hoc* analysis using Bonferroni's multiple comparison test. In some instances when ANOVA failed to reach significance (e.g., AUC analysis), a Student's t-test was employed to evaluate trends between groups utilizing Bonferroni's correction for multiple comparisons. Repeated measures ANOVA was used to determine significant differences between groups in the GTT. $p < 0.05$ was considered significant.

3. Results

3.1. Older, 9 and 13 MO, Tg2576 exhibit peripheral hyperinsulinemia

Following 16 hrs of fasting, insulin levels in serum samples from Tg2576 and WT littermates on control and RTZ-supplemented diet were measured. At 5 MO, no significant differences in fasted serum insulin levels were observed between Tg2576 mice and their WT littermates on control diet (one-way ANOVA; Fig. 1a). One-Way ANOVA showed that RTZ treatment did not significantly alter fasted insulin levels. By the time Tg2576 mice were 9 MO, serum insulin under fasted conditions was elevated compared to WT littermates. Treatment with RTZ lowered fasted serum insulin to a statistically equivalent level compared to WT values (Fig. 1b). One-Way ANOVA on insulin levels in 13 MO Tg2576 showed significantly higher levels in Tg2576 mice than in its WT littermates (Fig 1c). Similar to 9 MO mice, the hyperinsulinemia in 13 MO mice was reversed with one month RTZ treatment (Fig 1c).

3.2 No abnormalities in baseline fasted blood glucose

Following 16 hrs of fasting, we found that blood glucose levels for Tg2576 at all ages tested were not statistically different from WT mice, regardless of diet (one-way ANOVA; Fig 2a, b, c). Since hyperinsulinemia in both 9 and 13 MO Tg2576 mice was reversed by one month treatment with RTZ, these studies show that 9 and 13 MO Tg2576 suffer from hyperinsulinemia, but not hyperglycemia. The age-of-onset for hyperinsulinemia is around 9 MO; this abnormality was still evident at 13 MO.

3.3 Tg2576 exhibit age-selective dysregulation of peripheral glucose

To assess the insulin sensitizing effects of RTZ in our mice, we subjected the animals to a glucose tolerance test (GTT). GTT is a standard clinical test in which fasted (16 hrs) mice are challenged with a bolus of glucose, and insulin response is determined as a function of blood glucose levels during return to baseline. Upon glucose challenge, 5 MO Tg2576 mice exhibited a similar GTT response as 5 MO WT littermates (repeated measures one-way ANOVA, Fig 2a), indicating that insulin regulation in 5 MO Tg2576 mice is unaltered compared to WT mice. In addition, treatment with RTZ for 1 month did not affect GTT performance in response to glucose challenge in either genotype (Fig 2a). Area under the curve (AUC) analysis was used to evaluate insulin response in the GTT (Table 1). At 5 MO, AUC analysis confirmed statistically equivalent glucose clearance response in 5 MO WT and Tg2576, regardless of diet (one-way ANOVA, $p=0.36$).

As expected, two-way ANOVA analysis of the 5 MO GTT data demonstrated no interaction between genotype and treatment, suggesting that RTZ treatment did not alter the glucose response in animals with normal peripheral glucose, independently of their genotype.

At 9 MO, Tg2576 exhibited a different response to the glucose challenge compared to WT littermates. Baseline glucose levels were similar among genotypes and treatment groups at 9 MO. Nevertheless, 15 min after the glucose challenge, the Tg2576 mice on control diet had significantly higher blood glucose levels than WT and Tg2576 on RTZ treatment. Elevated glucose in untreated Tg2576 was maintained through 60 min after glucose challenge. Repeated measures ANOVA followed by Bonferroni *post hoc* analysis revealed that Tg2576 on control diet exhibited significantly higher blood glucose levels at the 15, 30 and 60 min time points (Fig 2b). One explanation for this observation is that the insulin response in WT (treated and untreated) and RTZ-treated Tg2576 mice leads to more effective glucose clearance between 0 and 60 min compared to untreated Tg2576. RTZ-treated 9 MO Tg2576 exhibited improved glucose clearance such that it mimicked WT littermates (Fig. 2b), indicating that RTZ reversed the abnormal peripheral glucose regulation.

Two-way ANOVA on 9 MO GTT data determined an interaction between genotype and treatment for time points 30, 60, and 90 min (Fig. 2b). This suggests that Tg2576 selectively responded to RTZ treatment through normalized response in the GTT.

Although one-way ANOVA analysis of 9 MO AUC data did not reach significance, Student's t-test with Bonferroni's correction for multiple comparisons demonstrates that untreated Tg2576 AUC was significantly higher than its WT littermates (Student's t-test; p -value = 0.01), providing additional evidence that 9 MO Tg2576 exhibit impaired glucose clearance in the GTT compared to WT littermates (Table 1). There were no statistical differences between RTZ-treated Tg2576 and WT on either control (Student's t-test; p = 0.08) or RTZ-supplemented diet (Student's t-test; p = 0.21) suggesting that one month RTZ treatment ameliorated 9 MO Tg2576 peripheral gluco-regulatory abnormalities.

At 13 MO, untreated Tg2576 group exhibited a different GTT response. At 90 and 120 min, Tg2576 mice have significantly lower blood glucose levels compared to WT mice (treated and untreated) and RTZ-treated Tg2576 (repeated measures ANOVA with Bonferroni's *post hoc* analysis, Fig. 2c). Nonetheless, RTZ treatment normalized Tg2576 GTT response; this suggests that 13 MO Tg2576 also exhibit peripheral glucoregulatory abnormalities (Fig. 2c). Two-way ANOVA analysis of 13 MO GTT data determined an interaction of genotype and treatment for time points 60 and 90 min suggesting that Tg2576 selectively responded to RTZ treatment through normalized response in the GTT. There were no statistical differences between RTZ-treated Tg2576 and WT littermates on either control or RTZ-supplemented diet.

AUC analysis of GTT data from the 13 MO groups did not reveal statistical significance with one-way ANOVA or Student's t-test for Bonferroni's correction for multiple comparisons (Student's t-test; p = 0.06; Table 1). Nonetheless, given that untreated Tg2576 exhibit elevated fasting glucose and altered glucose responses at later time points in the GTT, 13 MO Tg2576 clearly exhibit peripheral gluco-regulatory abnormalities.

Altogether our studies support the notion that Tg2576 mice exhibit dysregulation of peripheral glucose metabolism with age: 5 MO Tg2576 exhibit normal fasting insulin and normal response in the GTT; 9 and 13 MO fasting insulin levels are elevated and glucose responses in the GTT are altered compared to WT groups that are normalized with RTZ treatment.

3.4. RTZ reverses learning and memory deficits in 9 MO Tg2576

We next determined whether RTZ treatment led to beneficial effects on hippocampus-dependent cognitive deficits in Tg2576. As previously reported by our group, Tg2576 mice exhibit age-dependent decline in contextual fear learning (hippocampus-dependent), while cued fear learning (hippocampus-independent) remains intact. As we previously demonstrated, 5 MO Tg2576 mice exhibit decreased freezing behavior in the contextual test for fear conditioning compared to WT mice (Fig. 3) [14]. One-way ANOVA on freezing behavior in the contextual test for fear-conditioned learning revealed that RTZ treatment had no effect on 5 MO Tg2576's cognitive performance (Fig. 3a). However, treatment with RTZ was effective in 9 MO Tg2576 mice, as evidenced by contextual fear-conditioned learning performance that was statistically indistinguishable compared to WT mice on either control or RTZ treatment (one-way ANOVA, Fig. 3b). Thirteen MO Tg2576 mice showed cognitive impairment in contextual fear conditioned learning that was unresponsive to treatment with RTZ. One-way ANOVA found no effect of RTZ treatment on the cognitive performance of the mice at this age (Fig. 3c)

In summary, these studies demonstrate that one month treatment with RTZ given at 8 MO, but not 4 or 12 MO, is capable of reversing cognitive deficits in the Tg2576 AD mouse model.

4. Discussion

4.1 Insulin Modulates Cognition

It is well established that insulin plays a major role in regulating peripheral glucose levels. However, recent studies have found that insulin also plays a major role in brain metabolism as well as in modulating learning and memory. Insulin receptors are present in brain areas important for learning and memory function, such as the hippocampus and frontal cortex [28]. Insulin is known to modulate synaptic transmission and long-term potentiation [29,30]. In humans, intravenous insulin administration improves memory recall; in rodents, intracerebroventricular injection of insulin improves memory as well [31,32]

Clinical studies have found that AD patients exhibit decreased central insulin but elevated peripheral insulin levels when compared to age-matched control patients [33]. It has been hypothesized that elevated peripheral insulin saturates blood brain barrier insulin receptors, thus impairing insulin translocation into the brain [34]. This notion is supported by studies demonstrating that peripheral hyperinsulinemia leads to decreased insulin transport into the brain [35]. Our data suggest that further decline in cognitive performance in 9 MO Tg2576 correlates with the onset of peripheral gluco-regulatory abnormalities and hyperinsulinemia; dysregulation of insulin translocation from the periphery into the brain may be one factor contributing to these observations.

4.2 Hyperinsulinemia in Tg2576 is reversed by RTZ

Here we report that peripheral hyperinsulinemia is present in 9 and 13 MO Tg2576 but absent in 5 MO animals. These observations support previous reports of hyperinsulinemia in older Tg2576 under non-fasting conditions [22]. WT mice (at all ages studied) and 5 MO Tg2576 exhibit normal fasting insulin and treatment with RTZ had no effect on peripheral insulin in these animals. However, the hyperinsulinemia exhibited by 9 and 13 MO Tg2576 mice was normalized with one month RTZ treatment.

4.3 RTZ treatment normalizes peripheral glucose regulation in Tg2576 mice

The GTT is an indicator of proper peripheral insulin response. As was the case for fasting insulin measurements, all 5 MO mice responded in the GTT comparable to WT littermates. Neither peripheral insulin, nor baseline blood glucose, nor GTT response was affected by RTZ treatment in 5 MO WT or Tg2576. Hence, 5 MO Tg2576 do not exhibit measurable peripheral gluco-regulatory abnormalities.

Nine and 13 MO Tg2576 exhibit altered performance in the GTT further supporting the notion that aged Tg2576 mice exhibit gluco-regulatory abnormalities. This was exemplified by several time points at which glucose measurements were significantly altered compared to age-matched WT littermates; these differences were reflected in the overall AUC values as well. Our 9 MO GTT data correlate with previous studies in which it was reported that 8 MO Tg2576 mice have elevated blood glucose in response to injected insulin compared to WT littermates [22]. However, comparison of 9 MO and 13 MO Tg2576 shows that while both age groups present with abnormal glucose responses in the GTT, they are in opposite direction from each other. These divergent responses may be due to age-related mechanistic changes in peripheral insulin response. Perhaps the hyperinsulinemia phenotype in 13 MO Tg2576, which has existed for 5 months, is such that glucose clearance is accelerated due to augmented insulin response compared to 9 MO Tg2576. Nonetheless, aged (9 MO and 13 MO) Tg2576 exhibit a GTT response that is not typically seen in WT mice and one month treatment with RTZ normalized GTT performance.

Although 9 and 13 MO Tg2576 mice exhibit signs of peripheral gluco-regulatory abnormalities, such as elevated serum insulin, we did not interpret these levels to be severe since baseline glucose in Tg2576 mice at all ages were equivalent to age-matched WT littermates. Increased fasting insulin without an increase in fasting glucose suggests that the gluco-regulatory abnormalities of the Tg2576 mouse model are not equivalent to that of a T2DM animal model. For example, in order to determine if the animals exhibit classical insulin resistance we subjected our Tg2576 and WT littermates to an insulin tolerance test (ITT) by injecting a bolus of insulin (i.p 0.75U/kg) in overnight-fasted animals and measuring blood glucose [36]. In the ITT, Tg2576 glucose responses were indistinguishable from WT thus they cannot be classified as insulin-resistant or diabetic (data not shown). As demonstrated in several other publications, further manipulations, such as a high fat diet, are necessary to induce a more diabetic-like phenotype in these animal models [25,37].

4.4 Age-selective sensitivity to RTZ-mediated cognitive improvement

A correlation between metabolism and cognition has been shown in several AD mouse models [22–25]. For example the APP_{SWE} PSEN1dE9 mouse model that carries both mutant APP and presenilin-1 transgenes demonstrated impaired GTT performance and elevated fasting plasma insulin levels following chronic intake of sucrose-sweetened water [24]. In addition, Tg2576 insulin resistance accelerated by a high fat diet led to an exacerbation of cognitive deficits [25]. These observations clearly suggest that at certain ages, peripheral gluco-regulatory mechanisms can have a significant influence on cognitive function in AD mouse models.

However, simply reversing peripheral gluco-regulatory abnormalities does not appear to be sufficient to reverse cognitive deficits in these mice. We studied the age-selective efficacy of a one-month RTZ treatment regimen in reversing cognitive deficits observed in 4 to 13 MO Tg2576. After one month of treatment, each age group underwent an assessment of peripheral gluco-regulatory status and cognitive function. In our study, cognitive benefits with RTZ treatment were seen only in 9 MO Tg2576. Whereas both 9 and 13 MO Tg2576 treated for one month with RTZ responded with normalized serum insulin and GTT performance; cognitive function did not show any improvement in the 13 MO RTZ-treated Tg2576. Conversely, RTZ treatment was unable to improve learning and memory in 5 MO Tg2576 mice in which gluco-regulatory abnormalities are absent. As such, RTZ-mediated cognitive rescue does not correlate with peripheral gluco-regulatory status and likely reflects age-dependent mechanistic differences that underlie cognitive function in this AD model.

Previous studies have shown that age-dependent cognitive decline in Tg2576 undergo age-dependent mechanistic changes. For example, we have previously shown that calcineurin inhibition with FK-506, reverses cognitive deficits in 5 MO, but not in 12 MO Tg2576 mice [20]. These observations suggest that similar to the etiology of the human disease, the molecular mechanisms underlying progressive cognitive decline in Tg2576 change with age [27]. Likewise, the onset of cognitive deficits is independent of peripheral gluco-regulatory status since 5 MO Tg2576 are cognitively impaired yet exhibit normal insulin levels and normal responses in the GTT. In addition, peripheral gluco-regulatory abnormalities are not the decisive factor for RTZ efficacy on cognition since 13 MO Tg2576 have peripheral gluco-regulatory abnormalities that are responsive to one month of RTZ treatment but cognitive function is not rescued.

Based on the data presented herein, we hypothesize that simply correcting peripheral gluco-regulatory abnormalities does not guarantee cognitive improvement. In support of this are the findings of Nicolakakis et al., in which 14 MO Tg2576 mice were treated with pioglitazone, a related PPAR γ agonist, for 6–8 weeks [38]. While positive changes could be detected in cerebrovascular function, amyloidosis, and cholinergic function, this did not culminate in improved performance in a spatial learning and memory task. Furthermore, a study by Pedersen

et al. suggests that in order to achieve long-term effectiveness with RTZ treatment, treatment should be initiated around the onset of peripheral gluco-regulatory abnormalities [23]. In their study, RTZ treatment was initiated in 8 MO Tg2576 and maintained until 13 MO; this treatment regimen did result in improved learning and memory performance.

In summary, we propose that peripheral gluco-regulatory abnormalities in Tg2576 are not precipitating factors for loss of cognitive function but its manifestation at later ages likely contributes to the observed progression of cognitive decline with age (Fig. 4). Our data show that normalization of peripheral gluco-regulatory abnormalities with one month of RTZ treatment is not sufficient to ameliorate cognitive impairment in Tg2576 mice when begun long after the onset of peripheral gluco-regulatory abnormalities.

Acknowledgments

This work was supported by the National Institutes of Health under Ruth L. Kirschstein National Research Service Award (F31 NS052928) (J.R.R.), NIH Grant R01- AG031859, the Mitchell Center for Neurodegenerative Diseases, and the Sealy Foundation (K.T.D). Authors have disclosed no actual or potential conflicts of interest. We thank Dr. Dale Hogan, Mrs. Wanda Lejeune, and Dr. Wei Song for technical assistance, and Dr. Caterina M. Hernandez for editing assistance.

References

- 2009 Alzheimer's disease facts and figures. *Alzheimers Dement* 2009;5(3):234–270. [PubMed: 19426951]
- Larson EB, Kukull WA, Katzman RL. Cognitive impairment: dementia and Alzheimer's disease. *Annu Rev Public Health* 1992;13:431–449. [PubMed: 1599598]
- Forstl H, Kurz A. Clinical features of Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci* 1999;249(6):288–290. [PubMed: 10653284]
- Luchsinger JA, Tang MX, Shea S, Mayeux R. Hyperinsulinemia and risk of Alzheimer disease. *Neurology* 2004;63(7):1187–1192. [PubMed: 15477536]
- Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM. Diabetes mellitus and the risk of dementia: The Rotterdam Study. *Neurology* 1999;53(9):1937–1942. [PubMed: 10599761]
- Luchsinger JA, Tang MX, Stern Y, Shea S, Mayeux R. Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort. *Am J Epidemiol* 2001;154(7):635–641. [PubMed: 11581097]
- Xu WL, Qiu CX, Wahlin A, Winblad B, Fratiglioni L. Diabetes mellitus and risk of dementia in the Kungsholmen project: a 6-year follow-up study. *Neurology* 2004;63(7):1181–1186. [PubMed: 15477535]
- Arvanitakis Z, Wilson RS, Bienias JL, Evans DA, Bennett DA. Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. *Arch Neurol* 2004;61(5):661–666. [PubMed: 15148141]
- Craft S, Asthana S, Schellenberg G, Cherrier M, Baker LD, Newcomer J, Plymate S, Latendresse S, Petrova A, Raskind M, Peskind E, Lofgreen C, Grimwood K. Insulin metabolism in Alzheimer's disease differs according to apolipoprotein E genotype and gender. *Neuroendocrinology* 1999;70(2):146–152. [PubMed: 10461029]
- Jiang Q, Heneka M, Landreth GE. The role of peroxisome proliferator-activated receptor-gamma (PPARgamma) in Alzheimer's disease: therapeutic implications. *CNS Drugs* 2008;22(1):1–14. [PubMed: 18072811]
- Watson GS, Cholerton BA, Reger MA, Baker LD, Plymate SR, Asthana S, Fishel MA, Kulstad JJ, Green PS, Cook DG, Kahn SE, Keeling ML, Craft S. Preserved cognition in patients with early Alzheimer disease and amnesic mild cognitive impairment during treatment with rosiglitazone: a preliminary study. *Am J Geriatr Psychiatry* 2005;13(11):950–958. [PubMed: 16286438]
- Risner ME, Saunders AM, Altman JF, Ormandy GC, Craft S, Foley IM, Zvartau-Hind ME, Hosford DA, Roses AD. Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. *Pharmacogenomics J* 2006;6(4):246–254. [PubMed: 16446752]

13. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficits, A β elevation, and amyloid plaques in transgenic mice. *Science* 1996;274(5284):99–102. [PubMed: 8810256]
14. Dineley KT, Xia X, Bui D, Sweatt JD, Zheng H. Accelerated plaque accumulation, associative learning deficits and up-regulation of α 7 nicotinic receptor protein in transgenic mice co-expressing mutant human presenilin 1 and amyloid precursor proteins. *J Biol Chem*. 2002
15. Irizarry MC, McNamara M, Fedorchak K, Hsiao K, Hyman BT. APPSw transgenic mice develop age-related A β deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol* 1997;56(9):965–973. [PubMed: 9291938]
16. Irizarry MC, Soriano F, McNamara M, Page KJ, Schenk D, Games D, Hyman BT. A β deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. *J Neurosci* 1997;17(18):7053–7059. [PubMed: 9278541]
17. Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizarry M, Younkin L, Good MA, Bliss TV, Hyman BT, Younkin SG, Hsiao KK. Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat Neurosci* 1999;2(3):271–276. [PubMed: 10195221]
18. Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG. Age-dependent changes in brain, CSF, and plasma amyloid (β) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *J Neurosci* 2001;21(2):372–381. [PubMed: 11160418]
19. Westerman MA, Cooper-Blacketer D, Mariash A, Kotilinek L, Kawarabayashi T, Younkin LH, Carlson GA, Younkin SG, Ashe KH. The relationship between A β and memory in the Tg2576 mouse model of Alzheimer's disease. *J Neurosci* 2002;22(5):1858–1867. [PubMed: 11880515]
20. Dineley KT, Hogan D, Zhang WR, Taglialatela G. Acute inhibition of calcineurin restores associative learning and memory in Tg2576 APP transgenic mice. *Neurobiol Learn Mem* 2007;88(2):217–224. [PubMed: 17521929]
21. Taglialatela G, Hogan D, Zhang WR, Dineley KT. Intermediate- and long-term recognition memory deficits in Tg2576 mice are reversed with acute calcineurin inhibition. *Behav Brain Res* 2009;200(1):95–99. [PubMed: 19162087]
22. Pedersen WA, Flynn ER. Insulin resistance contributes to aberrant stress responses in the Tg2576 mouse model of Alzheimer's disease. *Neurobiol Dis* 2004;17(3):500–506. [PubMed: 15571985]
23. Pedersen WA, McMillan PJ, Kulstad JJ, Leverenz JB, Craft S, Haynatzki GR. Rosiglitazone attenuates learning and memory deficits in Tg2576 Alzheimer mice. *Exp Neurol*. 2006
24. Cao D, Lu H, Lewis TL, Li L. Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease. *J Biol Chem* 2007;282(50):36275–36282. [PubMed: 17942401]
25. Ho L, Qin W, Pompl PN, Xiang Z, Wang J, Zhao Z, Peng Y, Cambareri G, Rocher A, Mobbs CV, Hof PR, Pasinetti GM. Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. *Faseb J* 2004;18(7):902–904. [PubMed: 15033922]
26. Escribano L, Simon AM, Perez-Mediavilla A, Salazar-Colocho P, Del Rio J, Frechilla D. Rosiglitazone reverses memory decline and hippocampal glucocorticoid receptor down-regulation in an Alzheimer's disease mouse model. *Biochem Biophys Res Commun* 2009;379(2):406–410. [PubMed: 19109927]
27. McShane R, Areosa Sastre A, Minakaran N. Memantine for dementia. *Cochrane Database Syst Rev* 2006;(2):CD003154. [PubMed: 16625572]
28. Craft S, Watson GS. Insulin and neurodegenerative disease: shared and specific mechanisms. *Lancet Neurol* 2004;3(3):169–178. [PubMed: 14980532]
29. Watson GS, Bernhardt T, Reger MA, Cholerton BA, Baker LD, Peskind ER, Asthana S, Plymate SR, Frolich L, Craft S. Insulin effects on CSF norepinephrine and cognition in Alzheimer's disease. *Neurobiol Aging* 2006;27(1):38–41. [PubMed: 16298239]
30. Wickelgren I. Tracking insulin to the mind. *Science* 1998;280(5363):517–519. [PubMed: 9575095]
31. Craft S, Asthana S, Cook DG, Baker LD, Cherrier M, Purganan K, Wait C, Petrova A, Latendresse S, Watson GS, Newcomer JW, Schellenberg GD, Krohn AJ. Insulin dose-response effects on memory and plasma amyloid precursor protein in Alzheimer's disease: interactions with apolipoprotein E genotype. *Psychoneuroendocrinology* 2003;28(6):809–822. [PubMed: 12812866]

32. Park CR, Seeley RJ, Craft S, Woods SC. Intracerebroventricular insulin enhances memory in a passive-avoidance task. *Physiol Behav* 2000;68(4):509–514. [PubMed: 10713291]
33. Craft S, Peskind E, Schwartz MW, Schellenberg GD, Raskind M, Porte D Jr. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. *Neurology* 1998;50(1):164–168. [PubMed: 9443474]
34. Wallum BJ, Taborsky GJ Jr, Porte D Jr, Figlewicz DP, Jacobson L, Beard JC, Ward WK, Dorsa D. Cerebrospinal fluid insulin levels increase during intravenous insulin infusions in man. *J Clin Endocrinol Metab* 1987;64(1):190–194. [PubMed: 3536982]
35. Baura GD, Foster DM, Kaiyala K, Porte D Jr, Kahn SE, Schwartz MW. Insulin transport from plasma into the central nervous system is inhibited by dexamethasone in dogs. *Diabetes* 1996;45(1):86–90. [PubMed: 8522065]
36. Saha PK, Kojima H, Martinez-Botas J, Sunehag AL, Chan L. Metabolic adaptations in the absence of perilipin: increased beta-oxidation and decreased hepatic glucose production associated with peripheral insulin resistance but normal glucose tolerance in perilipin-null mice. *J Biol Chem* 2004;279(34):35150–35158. [PubMed: 15197189]
37. Kohjima M, Sun Y, Chan L. Increased food intake leads to obesity and insulin resistance in the tg2576 Alzheimer's disease mouse model. *Endocrinology* 2010;151(4):1532–1540. [PubMed: 20176720]
38. Nicolakakis N, Aboukassim T, Ongali B, Lecrux C, Fernandes P, Rosa-Neto P, Tong XK, Hamel E. Complete rescue of cerebrovascular function in aged Alzheimer's disease transgenic mice by antioxidants and pioglitazone, a peroxisome proliferator-activated receptor gamma agonist. *J Neurosci* 2008;28(37):9287–9296. [PubMed: 18784309]

Figure 1. Older, 9 and 12 MO, Tg2576 exhibit hyperinsulinemia

Tail blood was collected under fasting conditions. Samples were processed to obtain the serum, and serum insulin levels determined with LINCoplex kit (Millipore). **a**, 5 MO Tg2576 insulin levels were not significantly different from WT littermates. (one-way ANOVA $F(3,49)=1.33$; $p=0.28$). **b**, 9 MO Tg2576 demonstrated significantly higher insulin levels compared to WT littermates (one-way ANOVA $F(3,41)=4.11$; $p=0.01$). Treatment with RTZ significantly lowered serum insulin in Tg2576 mice ($p>0.05$) * indicates significance compared to WT on control diet; + indicates significance compared to Tg2576 on control diet). **c**, Similar to 9 MO Tg2576, 13 MO Tg2576 had significantly higher serum insulin levels than WT littermates (one-way ANOVA $F(3,81)=5.47$; $p=0.002$). Hyperinsulinemia in 13 MO Tg2576 was reversed

with RTZ treatment. * indicates significance compared to WT on control diet; + indicates significance compared to Tg2576 on control diet.

Figure 2. Tg2576 exhibit age-selective dysregulation of glucose

a, GTT performed on 5 MO Tg2576 (□) demonstrated normal glucose clearance, when compared to WT littermates (○) (one-way repeated measures ANOVA $F(3,15) = 3.17$; $p=0.06$). RTZ had no effect on the glucose clearance in neither, Tg2576 (▽) nor WT (△), genotype at 5 MO. **b**, 9 MO Tg2576 exhibit impaired glucose clearance compared to WT (one-way repeated measures ANOVA $F(3,15)=7.15$; $p=0.003$). One-way ANOVA revealed that Tg2576 on control diet exhibited significantly different glucose levels at the 15, 30 and, 60 min time points (15 min: $F(3,55) = 6.51$, $p=0.001$; 30 min: $F(3,55)=4.88$, $p=0.005$; and 60 min: $F(3,55)=2.72$, $p=0.05$). * indicates statistical significance [$p<0.05$] compared to WT. Treatment with RTZ normalized glucose clearance in 9 MO Tg2576 (one-way repeated measures ANOVA $F(3,15)$

=7.15; $p>0.05$). **c**, 13 MO Tg2576 performance in GTT is altered (one-way repeated measures ANOVA $F(3,15)=9.88$; $p=0.001$). One-way ANOVA revealed that Tg2576 on control diet have lower glucose levels at 90, and 120 min than WT (90 min: $F(3,46)=3.25$, $p=0.03$; 120 min: $F(3,46)=3.25$, $p=0.03$) * indicates significance compared to WT. Thirteen MO Tg2576 mice treated with RTZ cleared glucose similarly to WT treated and untreated (one-way repeated measures ANOVA $F(3,15)=9.876$; $p>0.05$).

Figure 3. RTZ reverses learning and memory deficits in 9 MO Tg2576

a, RTZ treatment did not rescue the contextual fear conditioning deficit in 5 MO Tg2576 (one-way ANOVA $F(3,16)=26.07$, $p<0.0001$). * indicates statistical significance compared to WT on control diet; ≠ indicates significance compared to WT treated with RTZ; + indicates significance compared to Tg2576 on control diet. **b**, Treatment with RTZ reversed the fear conditioning deficit in 9 MO Tg2576. (one-way ANOVA $F(3,16)=11.95$; $p<0.0001$; * indicates statistical significance compared to WT on control diet; ≠ indicates significance compared to WT treated with RTZ; + indicates significance compared to Tg2576 on control diet. **c**, RTZ treatment did not improve cognition in 13 MO Tg2576 (one-way ANOVA $F(3,16)=14.16$;

$p < 0.0001$; * indicates significance compared to WT on control diet; # indicates significance compared to WT treated with RTZ.

Figure 4. Schematic illustration of cognitive performance in Tg2576 and wild type littermate mice as a function of age

Based on our and others' work we can illustrate the cognitive decline exhibited by Tg2576 mice (gray solid line), compared to wild type mice (black solid line), over the lifespan of these animals [13,18–20]. One month RTZ treatment initiated around the onset of gluco-regulatory abnormalities (8 MO) reverses cognitive deficits in Tg2576 mice (gray dotted line). RTZ treatment that is initiated prior to manifestation of gluco-regulatory abnormalities (4 MO) or long after the onset of gluco-regulatory abnormalities (12 MO) are unsuccessful at rescuing cognitive function. These findings suggest a limited therapeutic window for effective RTZ treatment on cognition in Tg2576 AD mice.

Table 1

AUC values for GTT on 5, 9 and 13 MO Tg2576 and WT mice, on control or RTZ-supplemented diet

	5MO			9MO			13MO		
	AUC ^a	SEM ^b	n ^c	AUC ^a	SEM ^b	n ^c	AUC ^a	SEM ^b	n ^c
WT, Control Diet	26902	2420	18	17000	1334	10	25735	1626	15
Tg2576, Control Diet	25169	1590	14	23958*	2049	16	21709	1830	9
WT, RTZ Diet	25166	2398	15	21166	1926	18	25843	1623	14
Tg2576, RTZ Diet	25781	1670	20	19353	933	16	27553	1652	11

^a Average of area under the curve (AUC) for each group.

^b Standard error of mean (SEM).

^c Number of animals per group (n). See text for additional details and statistical analysis.

* indicates statistical significance (p<0.05) compared to WT littermates.