Milene Vandal,[1,2,3] Phillip J. White,[3,4,5,6] Cyntia Tremblay,[2] Isabelle St-Amour,[1,2,7] Geneviève Chevrier,[3,5] Vincent Emond,[2] Dominique Lefrançois,[1] Jessica Virgili,[1] Emmanuel Planel,[2] Yves Giguere,[8,9] Andre Marette,[3,5,6] and Frederic Calon[1,2,3]

Insulin Reverses the High-Fat Diet–Induced Increase in Brain Aβ and Improves Memory in an Animal Model of Alzheimer Disease

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Defects in insulin production and signaling are suspected to share a key role in diabetes and Alzheimer disease (AD), two age-related pathologies. In this study, we investigated the interrelation between AD and diabetes using a high-fat diet (HFD) in a mouse model of genetically induced AD-like neuropathology (3xTg-AD). We first observed that cerebral expression of human AD transgenes led to peripheral glucose intolerance, associated with pancreatic human Aβ accumulation. High-fat diet enhanced glucose intolerance, brain soluble Aβ, and memory impairment in 3xTg-AD mice. Strikingly, a single insulin injection reversed the deleterious effects of HFD on memory and soluble Aβ levels, partly through changes in Aβ production and/or clearance. Our results are consistent with the development of a vicious cycle between AD and diabetes, potentiating both peripheral metabolic disorders and AD neuropathology. The capacity of insulin to rapidly break the deleterious effects of this cycle on soluble Aβ concentrations and memory has important therapeutic implications.

Mounting evidence suggests that Alzheimer disease (AD), diabetes, and obesity are linked by their common reliance on insulin signaling pathways in the central nervous system (1). Besides its broad actions in the periphery, insulin in the brain regulates not only energy homeostasis, but also synaptic plasticity and memory function (2–5). Epidemiological studies revealed that dysfunction in insulin production and signaling is associated with poorer cognitive performance (6–8). Conversely, intranasal administration of insulin improves memory function when administered to cognitively impaired (9) or normal adults (10). Furthermore, type 2 diabetes (T2D), a condition characterized by impaired insulin homeostasis, is now recognized as an important risk factor for AD, which is the leading cause of cognitive dysfunction in the elderly (11–15).

While the interaction between such apparently distinct diseases is puzzling, it implies that treatments targeted toward boosting insulin signaling could tackle both AD and T2D at the same time. Therefore, intense efforts have been made in recent years to decipher causal mechanistic links underlying this association, particularly in animal models. One of the first clues comes from the demonstration that the induction of insulin resistance and diabetes results in an increase in Aβ production, tau phosphorylation, and cerebrovascular inflammation in the

[1]Faculté de Pharmacie, Université Laval, Québec, Canada
[2]Axe Neurosciences, Centre de Recherche du Centre Hospitalier de l'Université Laval, Québec, Canada
[3]Institut des Nutraceutiques et des Aliments Fonctionnels, Université Laval, Québec, Canada
[4]Sarah W. Stedman Nutrition and Metabolism Center, Duke Molecular Physiology Institute, Durham, NC
[5]Département de Medicine, Axe de Cardiologie, Faculté de Médicine de l'Université Laval, Québec, Canada
[6]Institut Universitaire de Cardiologie et de Pneumologie de Québec, Québec, Canada
[7]Département de Recherche et Développement, Héma-Québec, Québec, Canada
[8]Faculté de Médicine, Université Laval, Québec, Canada
[9]Centre de Recherche du Centre Hospitalier de l'Université de Québec, Hôpital Saint-François d'Assise, Québec, Canada

Corresponding author: Frederic Calon, frederic.calon@crchul.ulaval.ca.

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brain of several transgenic models of AD (16–19). Accordingly, increased postmortem microvascular pathology is found in AD patients with metabolic comorbidities, including diabetes (20). In contrast, several lines of evidence suggest that the clinical expression of AD includes metabolic impairment as well. Experiments performed with human postmortem brain tissue show that insulin signaling and the number of insulin receptors in the hippocampus are reduced in AD patients compared with age-matched control subjects (21,22). Moderate to severe AD patients also have lower cerebrospinal fluid insulin levels and reduced insulin-mediated glucose disposal when compared with healthy control subjects (23,24). Furthermore, early T2D-like biomarkers such as insulin resistance and glucose intolerance can be detected in AD mouse models, suggesting that AD pathology could also affect peripheral metabolism (25–29). However, it has yet to be determined whether metabolic deficits accelerate AD progression or whether AD causes defects in peripheral metabolism, or both.

We report in this study the presence of impaired glucose tolerance in a mouse model of genetically induced AD-like neuropathology (3xTg-AD), which progressed to pancreatic β-cell degeneration and impaired insulin production after exposure to a high-energy diet. The high-fat diet (HFD) also massively increased brain amyloid pathology and worsened memory function in 3xTg-AD mice. A single acute injection of insulin in 3xTg-AD mice fed with HFD restored memory function and brought back soluble brain Aβ concentrations to control levels, while increasing plasma Aβ levels in plasma. Our data thus reveal tight links between AD and peripheral metabolic defects and suggest that insulin signaling in the brain plays a key role in the production and clearance of Aβ from the brain to the blood.

RESEARCH DESIGN AND METHODS

Animals and Diets

All animal experiments were approved by the Laval University ethics committee. The 3xTg-AD (APP_{swe}, PSI_{M146V}, tau_{P301L}) mouse model of AD was used for the experiments (30). 3xTg-AD mice were produced at our animal facilities and compared with nontransgenic (NonTg) littermates with the same genetic background (C57BL6/129SvJ). For the dietary experiments, female mice received either a control diet (CD; 12% kcal fat) or an HFD (60% kcal fat) for a 9-month period starting at the age of 6 months (see ref. 17 for a detailed description of the diets). Females were selected because they exhibit higher AD-like pathology than males (31–33). All behavioral and metabolic testing were started 1 month before sacrificing the animals to minimize the impact of the anxiety generated by the tests and allow enough resting and acclimation time between the experiments. Animals were fasted for 6 h and received an intravenous injection of insulin (3.8 units/kg of human insulin) or saline 5 min before sacrifice. This dose is known to induce a robust activation of insulin signaling in the muscle and the liver (34). They were killed by intracardiac perfusion under deep anesthesia with ketamine/xylazine. Brain, pancreas, liver, epididymal adipose tissue, and gastrocnemius muscle were rapidly dissected and excised. Brain, muscle, and liver were kept at −80°C until processing for Western blot and ELISA analysis. The number of animals in each group for all experiments is indicated directly in the graph.

Insulin Sensitivity and Glucose Tolerance

Insulin sensitivity and glucose tolerance were assessed using intraperitoneal insulin tolerance tests (ITTs) and glucose tolerance tests (GTTs), respectively (n = 7–12 animals/group). Because we used female mice, all tests were performed during the diestrus phase of the estrus cycle to minimize hormonal variations during the tests. After 6-h fasting, mice were injected with 1 unit/kg human insulin or 1 g/kg glucose. Glycemia was measured with a glucometer (OneTouch UltraMini; LifeScan, Milpitas, CA) with a blood drop from the saphenous vein before the injection and 15, 30, 45, 60, 90, and 120 min after the injection.

Protein Extraction

The detailed procedure for protein extraction is found in the Supplementary Material and is described elsewhere (35).

Aβ40 and Aβ42 Quantification

Aβ40 and Aβ42 were measured in cortex soluble and insoluble fractions (n = 10–13) as well as plasma (n = 6–12) using a human β-Amyloid ELISA (Wako, Osaka, Japan) according to the manufacturer's instructions, and plates were read at 450 nm using a Synergy HT multidetection microplate reader (BioTek, Winooski, VT).

Histology

Epididymal fat and pancreas were dissected, weighed, and immediately postfixed in paraformaldehyde 4% (pH 7.4) for 48 h. Ten-micrometer sections were first hydrated in ethanol and stained in successive baths of hematoxylin (10 min), water (3 min), 70% alcohol, and 1% HCl solution and washed with running water. Lithium carbonate dips, running water, and eosin staining (30 s) were then performed prior to alcohol dehydration and coverslipping. Pancreatic islet area (n = 6 to 7 animals/group) was measured under bright-field illumination using Stereo Investigator software (MicroBrightField, Colchester, VT) integrated with an E800 Nikon microscope (Nikon Canada Inc., Mississauga, Ontario, Canada) from two sections of pancreas per mice. Approximately 20 islets per animal, from six to seven animals per group, were quantified with Neurolucida modeling software (MicroBrightField).

Western Immunoblotting

For Western immunoblotting, proteins from the parieto-temporal cortex were extracted with a Tris-buffered saline buffer for the soluble protein, a RIPA buffer for the membrane soluble protein, and a sarkosyl insoluble fraction for insoluble proteins, as described above. Protein quantification was done using bicinchoninic acid assays (Pierce, Rockford, IL). A total of 20 μg of protein from the cortex of 10–13 animals/group was loaded and separated

by SDS-PAGE and then electroblotted onto a polyvinylidene difluoride membrane (Immobilon; Millipore). Membrane were blocked in 5% milk with 0.5% BSA for 1.5 h and immunoblotted with primary and then secondary antibodies followed by chemiluminescence reagents (KPL, Gaithersburg, MD). The list of primary antibodies that were used in our experiments is available in the Supplementary Table 3. Intensity of the bands was assessed with a Kodak Image Station 4000MM Digital Imaging System (Molecular Imaging Software version 4.0.5f7; Kodak, New Haven, CT).

Plasma Insulin and C-Peptide Assay

Plasma insulin (n = 6–10 animals/group) and C-peptide (n = 5–8 animals/group) concentration were determined with an Ultrasensitive Insulin ELISA (Mercodia, Uppsala, Sweden) and C-peptide (Alpco Diagnostic, Salem, NH) according to the manufacturers' instructions.

Behavioral Assessment

Spatial and Recognition Memory Assessment

To evaluate recognition memory, the object-recognition task was performed (n = 9 to 10 animals/group). Mice were introduced in a clear box (29.2 cm × 619 cm × 612.7 cm) with two different objects for 5 min. One hour later, they were put in the same box with a new and a familiar object for 5 min. To verify the effect of insulin on recognition memory, a single insulin injection (1 unit/kg) was administered intraperitoneally, and the tests were performed 2 h later to allow glycemia to return to baseline (n = 6–13 animals/group). Recognition index = (time exploring the new object − time exploring the old object)/time exploring the old object. The time exploring the object was defined as the time the mouse spent smelling or exploring an object (36–38). Spatial memory evaluation was performed using the Barnes maze (San Diego Instruments, San Diego, CA) as previously described (39). The test lasted for 9 days, 4 training days (4 trials per day, 3 min each), and 2 test days (1 trial of 90 s). Mice were placed on a circular platform with 19 holes and 1 escape zone and exposed to two aversive stimuli: a bright light and a strong noise to stimulate the need to find the escape zone. The time to find the escape zone was used to evaluate the spatial memory of the mice.

Immunofluorescence

For cerebral immunofluorescence experiments, 25-μm–thick slices were used and stained with 6E10 antibody to label amyloid plaques. Plaque size was measured in the hippocampus at bregma −2.7 mm using the Stereo Investigator software (MicroBrightField) integrated with an E800 Nikon microscope (Nikon Canada Inc.) (n = 3–6 animals/group). For pancreatic immunofluorescence, paraffin-embedded 10-μm pancreas sections from mice perfused with paraformaldehyde (4%, pH 7.4) were used. Detection of amyloid pathology of the pancreatic tissue was done using 6E10 and 6C3 antibodies. To be able to quantify the number of 6E10-positive β-cells, a double staining was performed using an insulin antibody (n = 6 and 7 animals/group). The number of apoptotic β-cells in pancreatic islets was assessed with an antibody against cleaved caspase-3 (n = 5–9 animals/group). Anti-mouse or anti-rabbit secondary antibodies conjugated with either Alexa Fluor 488 or 568 were used. Immunofluorescence was examined using an epifluorescence microscope (Olympus Provis AX70; Olympus, Melville, NY) and photographs were taken using a SPOT digital camera (Diagnostic Instruments, Sterling Heights, MI). All images were prepared for illustration in Adobe Photoshop 7.0.

Statistical Analysis

Data are presented as mean ± SEM. Statistical analysis and number of mice per group are specified in each figure. Equality of the variances between the groups was determined using the Bartlett test. When more than two groups were compared, one-way or two-way ANOVA were used. For one-way ANOVA with equal variances groups, Tukey post hoc analysis was performed. For unequal variances, groups were compared with Dunnett post hoc analysis. When we had two groups to compare, an unpaired Student t test was performed, with a Welch correction included if variances were not equal. Coefficients of correlation were determined using the Pearson correlation test. Statistical significance was set at $P < 0.05$. All statistical analyses were performed with Prism 4 (GraphPad, San Diego, CA) or JMP (version 9.0.2; SAS Institute Inc., Cary, NC) softwares.

RESULTS

Feeding 3xTg-AD Mice With HFD Decreased Insulin Production and Worsened Memory Deficits

HFD Increases Body Weight and Reduces Insulin Sensitivity

To induce obesity and insulin resistance in 3xTg-AD mice, we fed them an HFD, from 6 months of age, known to trigger these metabolic perturbations and aggravate AD-like pathology (16,17). As expected, HFD induced a significant weight gain (32 and 42% vs. CD, NonTg and 3xTg-AD, respectively; Fig. 1*A*), higher calorie intake (17 and 13% vs. CD, NonTg and 3xTg-AD, respectively; Fig. 1*B*) and visceral fat accumulation (212 and 275% vs. CD, NonTg and 3xTg-AD, respectively; Fig. 1*C* and *D*), and a modest decrease in insulin sensitivity as evidenced by the ITT (82 and 70% ITT area under the curve [AUC]; Fig. 1*E* and *F*) and Western blot assessment of phosphorylated (p-)AKT (p-Ser^{473}) levels in the muscle (−5 and −24%) and liver (−36 and −55%) (Supplementary Fig. 1). However, HFD had a similar impact in both 3xTg-AD and NonTg mice on these parameters. Interestingly, 3xTg-AD transgenes alone were sufficient to induce glucose intolerance (120% compared with NonTg mice fed the CD).

HFD Potentiates the Effect of 3xTg-AD Transgenes on Glucose Tolerance

The HFD acted synergistically with 3xTg-AD transgenes to deteriorate glucose tolerance (28% compared with 3xTg-AD

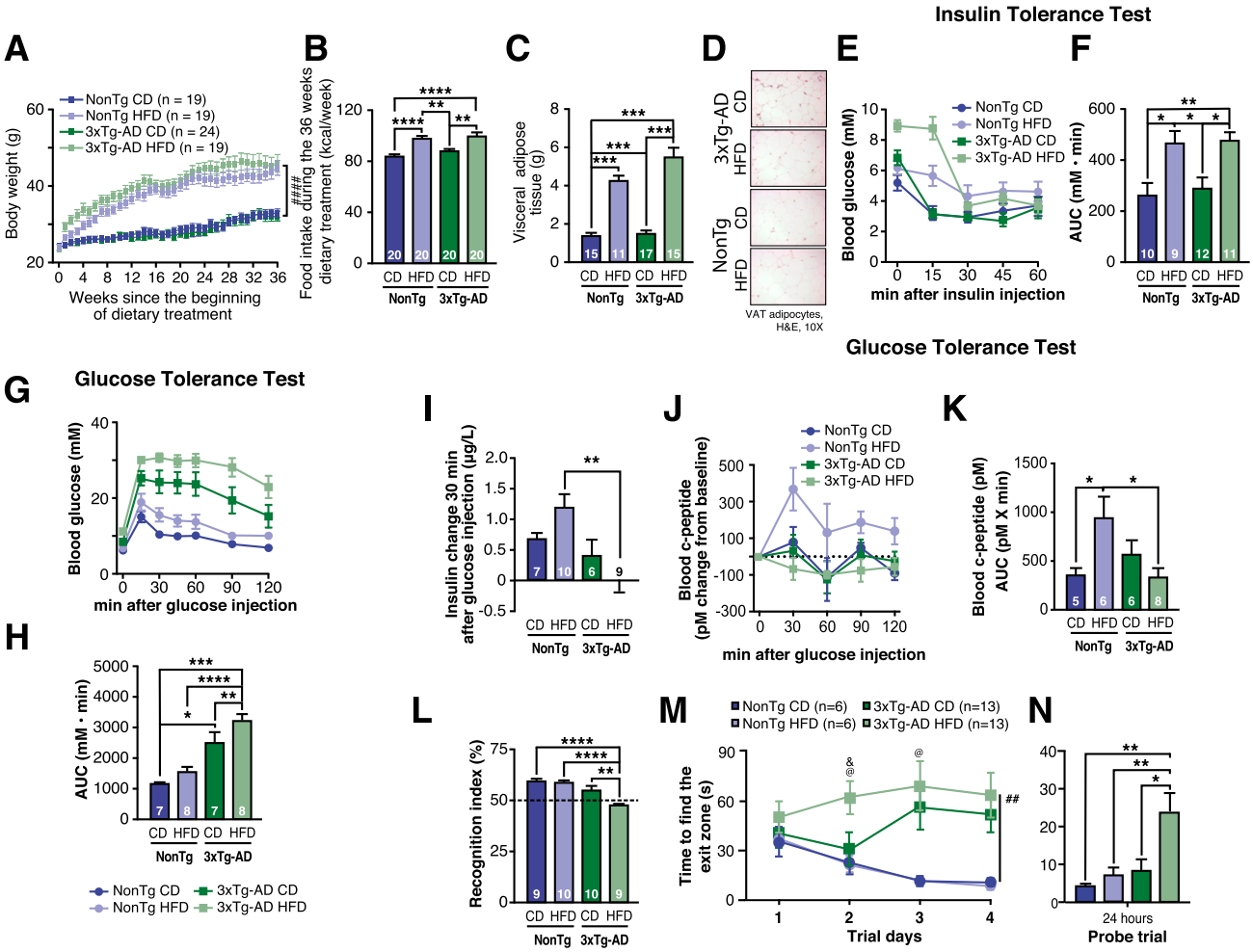

Figure 1—Diet-induced obesity combined with 3xTg-AD transgenes results in impaired insulin production, insulin resistance, and memory defects. *A*: Weekly body weight monitoring of 3xTg-AD and NonTg mice during the dietary treatment (from the age of 6 months). For body weight, AUC of the weight progression curves were compared. ####$P < 0.00001$, CD vs. HFD. *B*: Food intake. *C* and *D*: Visceral adipose tissue accumulation of mice after 9 months of diet. *C*: Visceral fat mass (epididymal and perirenal fat). *D*: H&E staining of visceral adipose tissue (VAT). *E* and *F*: Insulin sensitivity of 14-month-old mice (ITT). *G* and *H*: Glucose tolerance of 14-month-old mice (GTT). Insulin production during the GTT. *I*: Blood insulin. *J* and *K*: Blood C-peptide. *L–N*: Cognitive performance of 10-month-old mice. *L*: Object-recognition task (dotted line indicates a recognition index of 0.5). *M* and *N*: Barnes maze. Data are presented as mean ± SEM. Data are compared using one-way ANOVA and Tukey post hoc analysis. For Barnes maze, statistical comparison for trial days was made between mean AUC for each group of mice. ##$P < 0.01$, 3xTg-AD HFD vs. NonTg HFD; &$P < 0.05$, 3xTg-AD CD vs. HFD; @$P < 0.05$, 3xTg-AD HFD vs. NonTg HFD; *$P < 0.05$, **$P < 0.01$; ***$P < 0.0001$; ****$P < 0.00001$.

mice fed CD; Fig. 1*G* and *H*). As blood glucose levels are controlled through pancreatic insulin production, we measured insulin production (blood insulin and C-peptide) during the GTT. Insulin secretion after glucose administration was completely blunted in HFD-fed 3xTg-AD mice (−100% for insulin, Fig. 1*I*, and −55% for C-peptide, Fig. 1*J* and *K*), providing further evidence of an additive effect between HFD and AD transgenes on insulin production.

HFD Further Deteriorates Memory Function in 3xTg-AD Mice

We finally evaluated the impact of HFD on memory function in 10-month-old mice using two different tests: the object-recognition task (Fig. 1*L*) and the Barnes maze (Fig. 1*M* and *N*). Similar to what was observed in previous studies (16), both tests revealed further memory impairment in 3xTg-AD mice fed the HFD (−15% recognition index and 183% time to find the exit zone) without changes in voluntary locomotor activity or anxiety-like behavior (Supplementary Fig. 1).

3xTg-AD Pancreatic Islets Accumulate Human Aβ Peptide

Human Amyloid Pathology in the Pancreas of 3xTg-AD Mice

Insulin is produced by pancreatic β-cells. A shared pathological hallmark of T2D and AD is the presence of amyloid pathology (40), which, in T2D, is associated with apoptosis and loss of β-cells (41). The 3xTg-AD mouse model expresses an amyloid precursor protein (APP) bearing the Swedish mutation under the control of the Thy 1.2 promoter, restricting transgene expression and human

Aβ production in cerebral tissue (30,42). We thus sought to determine whether Aβ accumulated in the pancreas as well using an immunofluorescence technique with the 6E10 antibody raised against a protein sequence found in both human APP and Aβ but not in murine orthologs. Indeed, human APP/Aβ staining was found in the pancreas of 3xTg-AD mice, where it was localized in the islets (Fig. 2*A*). A semiquantitative analysis revealed that ~90% of islets were 6E10 positive in 15-month-old 3xTg-AD mice, with the prevalence of 6E10-positive islets similar between control and HFD-fed mice (Fig. 2*B*). As the 6E10 antibody targets, both Aβ and full-length APP of human origin, we used another antibody to bind specifically to human Aβ peptides. Consistently, the Aβ-specific staining was apparent only in 3xTg-AD mice (Fig. 2*C*). The absence of pancreatic expression of APP or tau transgenic mRNA confirmed that the Aβ peptide found in the pancreas was not derived from APP expressed locally (Fig. 2*D*).

3xTg-AD Transgenes and HFD Are Associated With Pancreatic Islet Degeneration

As the Aβ peptide has been shown to induce apoptosis markers in brain cells (43,44), we examined pancreatic islets for caspase-3 activation. 3xTg-AD mice fed the HFD had 116% more islets that stained positive for cleaved caspase-3 than HFD-fed NonTg mice (Fig. 2*E* and *F*), suggesting that Aβ might potentiate the effect of HFD on β-cell death (45). These observations were corroborated by islet areas 58% smaller in the pancreas of 3xTg-AD mice fed the HFD compared with NonTg mice on the same diet (Fig. 2*G* and *H*). Taken together, these results suggest that Aβ peptides produced in the brain were transported to the periphery and accumulated in pancreatic β-cells, triggering islet degeneration and impairing insulin production.

Insulin Restores to Control Levels the HFD-Induced Increase in Soluble Amyloid Pathology

Massive Increase in Cortical Soluble Aβ Concentrations After 9 Months of HFD

The induction of obesity in the 3xTg-AD mouse not only caused a deterioration of memory function, but also had peripheral pathological consequences including impaired insulin production. We first determined the extent by which the HFD would increase AD neuropathology in our experimental paradigm. While a 4-month exposure to the HFD had overall no effect on amyloid or tau protein levels in 10-month-old mice (Supplementary Fig. 2), prolonging the diet to 9 months led to a massive increase in soluble Aβ40 and Aβ42 in the cerebral cortex of 15-month-old 3xTg-AD mice (536%) (Fig. 3*A*), in line with previous studies (16,17).

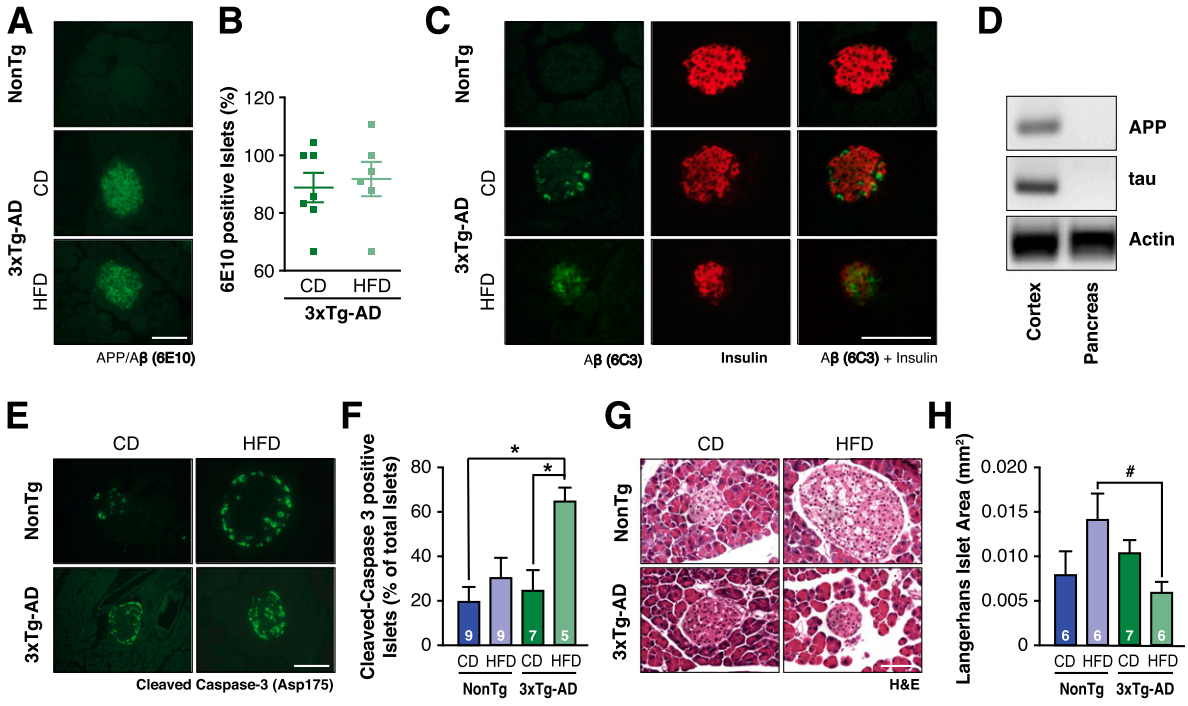

Figure 2—3xTg-AD mouse pancreatic islets accumulate human amyloid pathology. *A* and *B*: 6E10 staining and quantification of pancreatic islet pathology (19 islets/animal). *C*: Immunofluorescence of insulin (red) and Aβ (green) in pancreatic islets. *D*: Human APP and tau transgene expression in pancreatic and cortical tissue (n = 4) by RT-PCR. *E* and *F*: Cleaved caspase-3 immunostaining and quantification (13 islets/animal). H&E staining of pancreatic islets (*G*) and pancreatic islets mean area (*H*) (20 islets/animal). Mice were fed with HFD or CD from the age of 6 months. Data are presented as mean ± SEM. Data are compared using one-way ANOVA followed by a Tukey post hoc analysis. For pancreatic islets area, data from HFD-fed NonTg and 3xTg-AD mice were compared using an unpaired Student t test. *P < 0.05. #P < 0.05.

Insulin Reduces Cortical Soluble Aβ Concentrations in HFD-Fed 3xTg-AD Mice

To determine whether such an accumulation of soluble Aβ was caused by a deficit in brain insulin signaling (26,46,47), groups of animals were challenged with an acute insulin intravenous administration 5 min before their death. We first measured tau protein levels and found no major effect of insulin of soluble phosphorylated tau in control or HFD-fed mice (Supplementary Fig. 3). Nevertheless, we found that the level of sarkosyl-insoluble tau was reduced following insulin injection (Supplementary Fig. 3, −20 and −27%). Yet, insulin increased cortical p-AKT (p-Ser^{473}) by 30% in 3xTg-AD mice cortex (Supplementary Fig. 3), indicating a detectable effect on the phosphoinositide 3-kinase–AKT cellular cascade. No effect of insulin was seen on synaptic protein concentrations (Supplementary Fig. 3 and Supplementary Table 1). Strikingly, insulin injection completely reversed the HFD-induced rise in cortical soluble Aβ levels back to control levels in the cortex of 3xTg-AD mice (−86%) (Fig. 3*A* and *B*). Similar results were observed in the hippocampus (Supplementary Fig. 2). No detectable changes were observed in insoluble Aβ40 (Fig. 3*C*) and Aβ42 (Fig. 3*D*) or plaque formation (Fig. 3*F* and *G*). Accordingly, a single insulin injection decreased the soluble/insoluble Aβ42 ratio (Fig. 3*E*).

Insulin Improves Memory Function in HFD-Fed 3xTg-AD Mice

Because of the known effects of insulin on synapse formation and synaptic remodeling (48,49), we measured the behavioral consequences of a single intraperitoneal insulin injection. In line with previous data generated from intracerebroventricular insulin in rats (50), we found that memory function, as measured by the object-recognition index, was improved by 38% 2 h after a single intraperitoneal insulin injection in 16-month-old mice fed the HFD for over 10 months (Fig. 3*H* and *I*; $P < 0.05$). Therefore, the rise in soluble Aβ concentrations occurring after several months of exposure to HFD in 3xTg-AD mice could be rescued to control levels by a single administration of insulin, which also restored memory impairment.

Insulin Decreases Aβ Production and Increases Aβ Clearance

Insulin Reduces Aβ Production

We next sought to find plausible mechanisms underlying the effects of insulin on amyloid pathology and memory in the 3xTg-AD mouse. We first evaluated Aβ production by indexing the amyloidogenic cleavage of APP. Despite no difference in full-length APP (Fig. 4*A*) or β–C-terminal fragment (Supplementary Fig. 4), intravenous insulin administration increased soluble α-APP by 69% in mice fed the CD and by 154% in mice fed the HFD within 5 min (Fig. 4*B*). We then identified three molecular correlates, which could underlie the insulin-induced decrease in Aβ levels and the increase in α-APP. First, a reduction in β-secretase 1 (BACE1) protein was detected in insulin-injected 3xTg-AD mice (−29% CD and −53% HFD) (Fig. 4*D*). BACE1 cleaves the extracellular fragment of

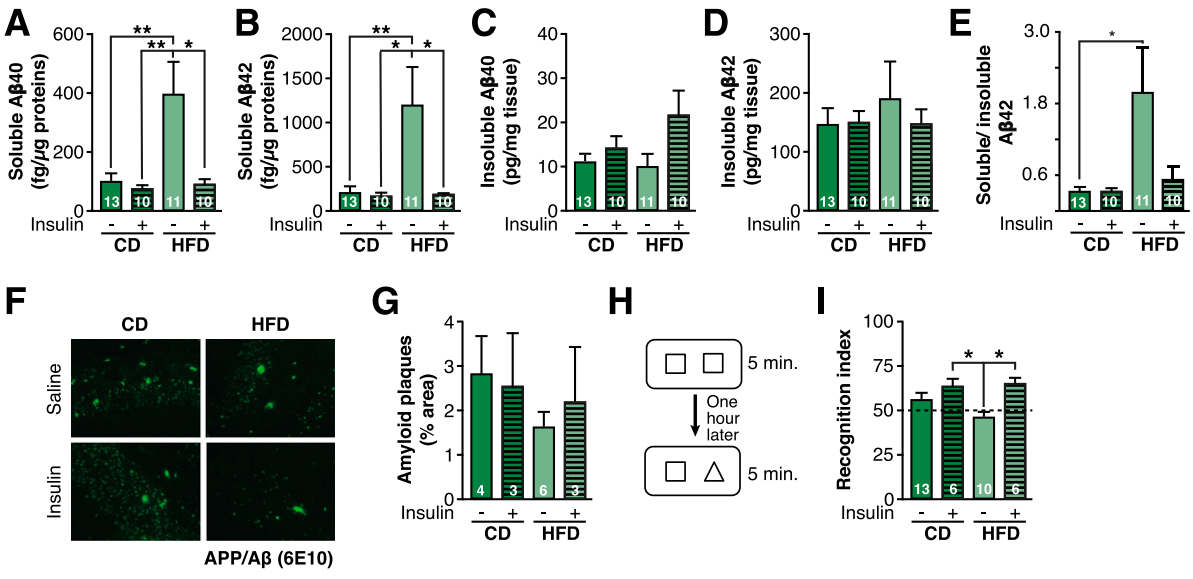

Figure 3—Insulin improves object recognition and reverses the HFD-induced increases in Aβ concentrations in the brain. Parieto-temporal cortex amyloid pathology of 3xTg-AD mice fed the CD or HFD, from the age of 6 months, for 9 months 5 min after a single intraperitoneal insulin (3.8 units/kg) or saline injection. *A* and *B*: Soluble amyloid protein. Insoluble amyloid protein levels (*C* and *D*), soluble/insoluble Aβ42 ratio (*E*), and amyloid plaques load (*F* and *G*) in cerebral cortices of mice. *H* and *I*: Memory function of 3xTg-AD mice fed the CD or HFD for 10 months, from 6 months of age, 2 h following insulin injection (1 unit/kg) (object-recognition task). Dotted line indicates a recognition index of 0.5. Data are presented as mean ± SEM. To compare all groups separately, data are compared using one-way ANOVA followed by a Tukey post hoc analysis when variances were equal variances. A Dunnett post hoc analysis was performed to compare groups when variances were not equal. *$P < 0.05$; **$P < 0.01$.

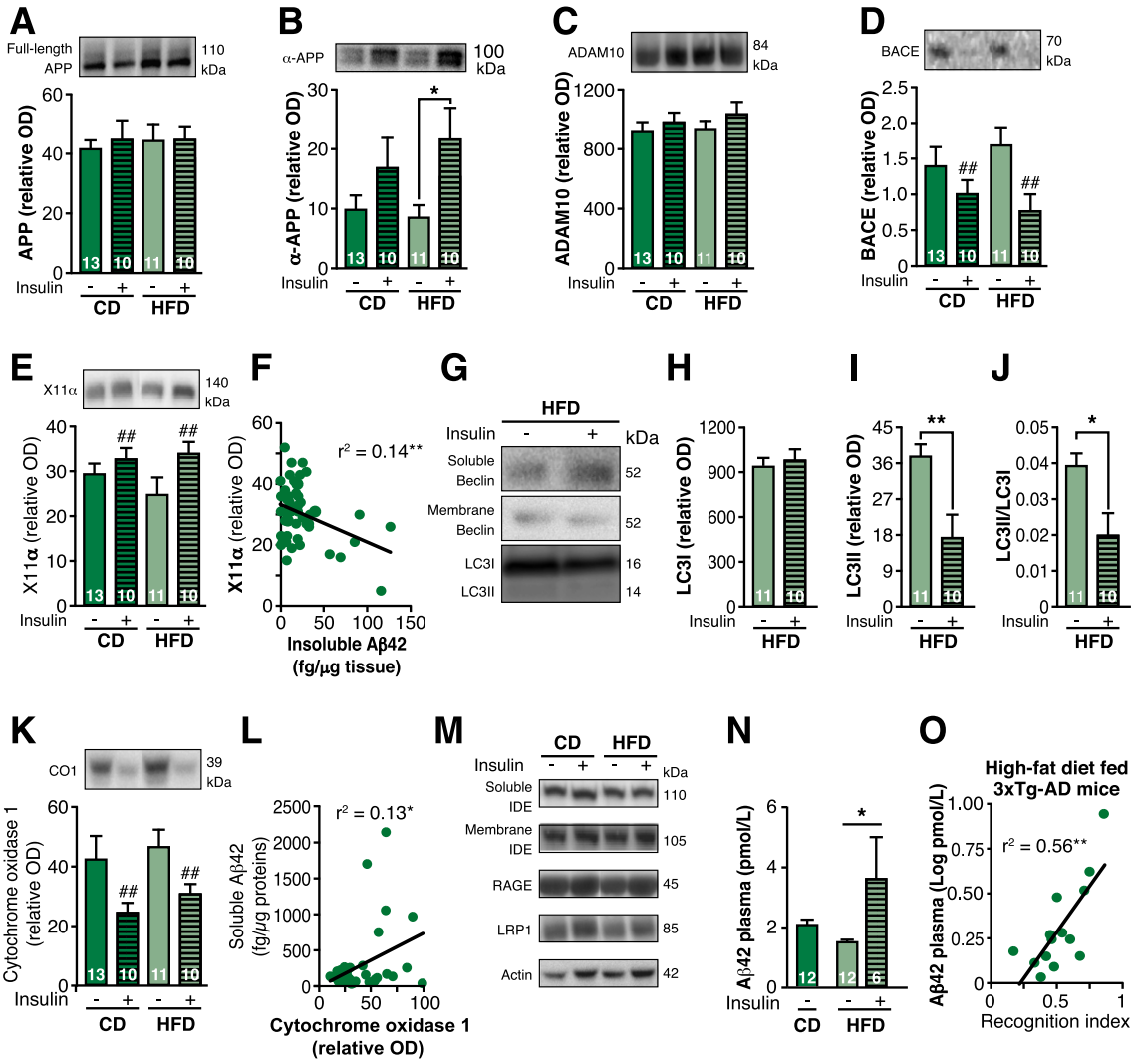

Figure 4—Insulin modulates Aβ production and clearance. Parameters involved in the production of Aβ peptides. Full-length APP (*A*), α-APP (*B*), α- (*C*) and β-secretase (BACE) (*D*), X11α protein (*E* and *F*), autophagy-related protein (*G–J*), and CO1 protein (*K* and *L*) in the cortex of 3xTg-AD mice fed the HFD for 9 months, from the age of 6 months, and intravenously injected with insulin (3.8 units/kg) or saline 5 min before sacrifice. *M–O*: Aβ clearance. *M*: Proteins implicated in the clearance of soluble Aβ in the cortex of 3xTg-AD mice fed the HFD for 9 months injected with insulin (3.8 units/kg) or saline 5 min before sacrifice. *N* and *O*: Plasma Aβ42 concentrations in mice fed with CD or HFD for 10 months were measured 15 min following insulin (1 unit/kg) or saline administration. Data are presented as mean ± SEM. To compare all groups separately, one-way ANOVA followed by a Tukey post hoc analysis was performed for equal variances. A Dunnett post hoc analysis was performed to compare groups when variances were not equal (plasma Aβ42 concentration). To verify the effect of insulin and dietary treatment, two-way ANOVA was performed. r^2 were determined using Pearson correlation test. Unpaired *t* test was used when two groups were compared. ##$P < 0.01$; *$P < 0.05$; **$P < 0.01$. ADAM10, α-secretase.

APP, which is later cleaved by the γ-secretase complex generating at the same time Aβ peptide (51). This observation is supported by previous in vitro studies showing a decrease BACE concentration following addition of insulin to cell culture (52). Second, insulin administration increased X11α (14% CD and 37% HFD), an adaptator protein that binds to APP and reduces its amyloidogenic cleavage (53,54), which was inversely correlated with insoluble Aβ42 (r^2 = 0.13) (Fig. 4*E* and *F*). Third, insulin reduced by 50% the LC3I/LC3II ratio in mice fed the HFD (Fig. 4*G–J*), consistent with a reduction of autophagy. As β-secretase cleavage can occur in the autophagosome, reduction of autophagy could be another mechanism by which insulin decreases Aβ production (55). Because recent research revealed that amyloid pathology is rapidly regulated accordingly to brain metabolic status (56,57), we determined the concentration of subunit 1 of cytochrome oxidase (CO1), which is considered a reliable index of metabolic status (58). Yet, we found a 42 and 33% reduction of the protein level of CO1 in the cerebral cortex of mice fed either the CD or HFD, respectively, following insulin injection (Fig. 4*K*). Interestingly, cortical CO1 was positively correlated with soluble Aβ42 concentrations (r^2 = 0.13) (Fig. 4*L*). Altogether, these data are

consistent with an insulin-induced reduction in Aβ production. However, it is unlikely that such a metabolic process alone explains the massive reduction of soluble amyloid protein observed in 3xTg-AD mice within 5 min after insulin injection.

Insulin Increases Aβ Clearance

Another key factor underlying the pathogenic accumulation of Aβ in the brain is its clearance from cerebral tissue (59,60). We first found no change in levels of insulin-degrading enzyme (Fig. 4*B* and Supplementary Table 2), the main Aβ-degrading enzyme (61–63). Secondly, we studied two transporter proteins implicated in the influx and efflux of Aβ across the blood–brain barrier (64–66): LDL receptor-related protein 1 and receptor for advanced glycation end products (Fig. 4*M* and Supplementary Table 2). However, no effect of insulin was detected on the concentrations of these proteins in the cortex of 3xTg-AD mice. Since part of brain Aβ is cleared toward the blood (60,67), we measured plasma Aβ42 following i.v. insulin injection. Interestingly, a 2.4-fold increase in Aβ42 was detected 15 min following insulin injection in mice fed with HFD for 10 months (Fig. 4*N*). This is in agreement with data in human AD patients in which insulin injection results in increased plasma Aβ within 120 min (68,69). Interestingly, plasma Aβ42 concentrations were positively correlated with the performance of 3xTg-AD mice in the object-recognition task (r^2 = 0.56) (Fig. 4*O*). In brief, our results suggest that the rapid reversing effect of insulin on soluble Aβ in the brain results from of a combination of complementary mechanisms acting on central Aβ production and clearance toward the blood.

DISCUSSION

We used the 3xTg-AD mouse model of AD to study the interrelation of two highly prevalent diseases in the elderly, AD and T2D. This strategy allowed us to demonstrate that the genetic induction of AD-like pathology in the brain was sufficient to induce peripheral glucose intolerance in mice, but not insulin resistance. Our data pinpointed the accumulation of toxic Aβ in the islets as a possible cause of a defect in pancreatic insulin production. The introduction of an obesogenic diet induced insulin resistance in both NonTg and 3xTg-AD mice, but in the latter, it exacerbated not only amyloid pathology but also diabetic signs such as pancreatic β-cell death and insulin secretion failure. Such defects in insulin production and signaling had major consequences on AD-like phenotype, as a single injection of insulin restored soluble Aβ concentrations to control level, coinciding with increased plasma Aβ concentrations and improved memory in the object-recognition task.

Synergistic Effects of HFD and AD-Like Pathology on Glucose Intolerance and Islet Degeneration

The glucose intolerance observed in the 3xTg-AD mouse model of AD was particularly striking and is in general agreement with previous observations made in other models of cerebral amyloidosis (26,28,70). Feeding 3xTg-AD mice with an obesogenic diet further decreased glucose tolerance and blunted the secretion of insulin and C-peptide in plasma after acute glucose administration. This observation suggests that glucose intolerance in the 3xTg-AD mouse results from a defect in insulin production in the pancreas. In the same animals, we observed a massive accumulation of human Aβ in pancreatic β-cells associated with a notable shrinkage of islets and signs of caspase activation. Because APP/tau transgenes are expressed under a neuronal promoter (30), consistent with our PCR analyses, the most probable source for Aβ found in this study in pancreatic β-cells is the brain. This raises the intriguing possibility that Aβ peptides found in pancreatic cells derive from circulating Aβ originating from the brain. As Aβ and amylin share a common pathological pathway (71), the presence of Aβ peptide in the pancreas of 3xTg-AD may have damaged pancreatic β-cells. Such a hypothesis is fueled by the fact that amyloid pathology is associated with degeneration of islet cells in the pancreas of diabetic patients (71). Index of caspase activation and hypomorphic pancreatic islets found in 3xTg-AD mice fed the HFD also support this idea. Thus, consequent defects in pancreatic β-cell function could account, at least in part, for the reduction of insulin production in 3xTg-AD mice fed the HFD.

A Key Role of Insulin in the Modulation of Cerebral Aβ Concentrations

A particularly surprising result presented in this study is the rapid ability of insulin to decrease soluble brain Aβ levels. Within minutes, intravenous insulin administration cancelled the long-term effect of the HFD on soluble Aβ concentrations in the cortex and hippocampus of 3xTg-AD mice. To our knowledge, this is the first evidence for such a rapid effect of a treatment on soluble Aβ. Our subsequent investigations identified changes in molecular markers implicated in Aβ production, all altered by a single insulin injection, including increased α-APP, increased X11α, decreased BACE, and decreased autophagy-related proteins (53,55,72). Brain soluble Aβ concentration is known to depend on neuronal activity (56,68,73), possibly through changes in amyloidogenic APP processing (68). Such an effect can be rapid, as microdialysis data show that lowering neuronal activity decreases Aβ levels within a 1–4-h time frame in vivo (56). The present data suggest that insulin may act through these metabolic pathways since it acutely induced a reduction of CO1, a well-established marker of neuronal activity, which was correlated with Aβ42 levels. Together and in line with in vitro data (52), our results support an effect of insulin on Aβ production.

Through regulation of Aβ steady-state levels in the brain, Aβ clearance from the brain to cerebrospinal fluid and blood is believed to play a key role in the progression of late onset AD (74). We found that the decrease in Aβ levels in the brain coincided with a sharp rise in Aβ in plasma of 3xTg-AD mice following insulin injection. This suggests that the potentiation of Aβ clearance is the

mechanism most likely to explain such a rapid insulin-induced reduction in brain Aβ concentrations. Therefore, our results argue for pleiotropic effects of insulin on complementary pathways, leading to decreased Aβ production and increased Aβ clearance within minutes.

Insulin, A Potential Therapeutic Tool in AD

The rapid effect of insulin on soluble Aβ levels observed has several implications. First, such quick variation induced by an endogenous peptide hormone calls for caution when using soluble Aβ concentrations as long-term surrogate markers of AD progression, in neuroimaging or as a biomarker in biological fluids (75). Still, that soluble Aβ varies so quickly in the brain is not so surprising based on recent data in humans indicating that soluble Aβ concentrations oscillate through the day and according to brain metabolic status (56,72,75,76). Nevertheless, the effect of insulin on brain and plasma Aβ was accompanied by improved memory performance in object recognition, supporting a therapeutic relevance of insulin action. Despite the possibility that insulin may have acted through mechanisms unrelated to Aβ, the coincidence is intriguing. Although such a rapid effect of insulin on Aβ concentrations needs first to be demonstrated in humans, our data strengthen the case already made by several authors (1,24,77) of using insulin as a therapeutic tool in AD. Because of the risk of hypoglycemia and the possible induction or exacerbation of peripheral insulin resistance, caution is warranted as the effects observed in this study follow an acute injection and may not be sustainable over a chronic treatment. However, intranasal administration could be an interesting alternative to deliver insulin to the brain, in the light of encouraging preliminary results in AD patients (77).

Taken together, our results suggest that in the 3xTg-AD mouse model of AD, centrally expressed Aβ can contribute to pancreatic cell degeneration in both AD and T2D, leading to defects in insulin production. Exaggerated dietary intake of fat-derived energy could trigger a vicious cycle for which the main consequences are further accumulation of Aβ in the brain and impaired cognition, which can be reversed by a single injection of insulin. If replicable in humans, these findings highlight the potential of correcting insulin signaling defects as a promising therapeutic tool to modulate cerebral concentrations of Aβ and treat Aβ-related symptoms of AD.

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