Title: Alzheimer’s amyloid-β oligomers induce hypothalamic inflammation and peripheral insulin resistance in mice


Affiliations:
1 Institute of Medical Biochemistry, and 2 School of Pharmacy, Federal University of Rio de Janeiro, Rio de Janeiro, RJ 21944-590, Brazil
3 Department of Internal Medicine, Faculty of Medical Sciences, State University of Campinas, Campinas, SP 13484-350, Brazil
4 Department of Neurobiology, Northwestern University, Evanston, IL 60208, USA
5 Center for Neuroscience Studies, Queen’s University, Kingston, ON K7L 3N6, Canada.

*Correspondence to: Fernanda G. De Felice and Sergio T. Ferreira, Institute of Medical Biochemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ 21944-590, Brazil; Emails: felice@bioqmed.ufrj.br; ferreira@bioqmed.ufrj.br
Abstract: Alzheimer’s disease (AD) is increasingly associated with peripheral metabolic disorders. Clinical data indicate increased risk of diabetes in AD patients. Early studies showed that AD brains exhibit deposits of amyloid-β peptide (Aβ) in the hypothalamus, a brain region involved in metabolic regulation and extensively affected in type 2 diabetes and obesity. However, it is unknown whether and how Aβ oligomers (AβOs), toxins causing early neuronal dysfunction in AD, target and affect the hypothalamus. Here, we show that infusion of AβOs into the lateral cerebral ventricle of mice and monkeys activated the pro-inflammatory IKKβ/NF-κB pathway and caused transient endoplasmic reticulum (ER) stress in the hypothalamus. The impact of AβOs on the hypothalamus was followed by deregulated peripheral glucose homeostasis and feeding behavior, impaired insulin signaling in skeletal muscle and adipose tissue inflammation in mice. AβOs failed to trigger hypothalamic IKKβ activation and peripheral glucose intolerance in tumor necrosis factor α (TNFα) receptor 1 knockout mice. Pharmacological inhibition of brain ER stress rescued peripheral glucose homeostasis and prevented increased sympathetic tonus caused by AβOs. Results reveal a novel pathogenic action of AβOs in the brain leading to deregulated peripheral glucose homeostasis, demonstrating shared molecular mechanisms between hypothalamic dysfunction in metabolic disorders and AD.
Introduction

Increasing evidence suggests an association between metabolic disorders, notably Type 2 Diabetes (T2D), and Alzheimer’s disease (AD) (1;2). Clinical and epidemiological studies indicate that diabetic patients have increased risk of developing AD (3-5) and AD brains exhibit defective insulin signaling (6-9). Recent studies have shown that amyloid-β peptide oligomers (AβOs), toxins that build up in AD brains and have been proposed to be major players in synapse failure in AD (reviewed in (10-12), are linked to impaired hippocampal insulin signaling. AβOs were found to cause internalization and cellular redistribution of insulin receptors and to block downstream insulin signaling in hippocampal neurons (6;13-15).

Hyperinsulinemic/hyperglycemic individuals and mice show increased plasma and brain levels of Aβ (16-18), suggesting that altered peripheral metabolic homeostasis may increase Aβ levels and influence AD development (1).

Intriguingly, AD has further been associated with increased risk of T2D development (19), suggesting that the AD-T2D connection may be a two-way road. Early studies demonstrated peripheral glucose intolerance in AD patients (20). Recently, hyperglycemia and hyperinsulinemia, cardinal features of T2D and other metabolic syndromes, were found to positively correlate with development of AD-like brain pathology in humans (21), and obesity-induced insulin resistance is exacerbated in transgenic mouse models of AD (17;22). However, the molecular mechanisms underlying these observations are still largely unknown.

The hypothalamus plays a key role in neuroendocrine interaction between the central nervous system and the periphery (23;24). Emerging evidence further indicate that hypothalamic inflammation and endoplasmic reticulum (ER) stress are critical pathogenic events in the establishment of peripheral insulin resistance in metabolic disorders (25-28). An interesting recent study showed that hypothalamic inflammation accelerates ageing and shortens lifespan in mice (29). Interestingly, early postmortem analysis of AD brains identified Aβ deposits in the hypothalamus (30;31). Moreover, voxel-based morphometry revealed reduced hypothalamic volume in early AD compared to healthy controls (32), and a decrease in the number of hypothalamic orexin neurons was recently reported in AD brains (33). While the hypothalamus has been largely ignored in the AD field, these studies indicate that this brain region may indeed
be affected in AD. We hypothesized that AβOs, recently shown to induce inflammation, ER stress and defective insulin signaling in the hippocampus (6;34), could also target the hypothalamus, and that AβO-induced hypothalamic deregulation might be linked to impaired peripheral glucose homeostasis in AD.

We show that AβOs bind to primary hypothalamic neurons in vitro and accumulate in hypothalamic neurons in the brains of cynomolgus monkeys given intracerebroventricular (i.c.v.) infusions of AβOs. AβOs further triggered aberrant generation of reactive oxygen species (ROS) in cultured hypothalamic neurons, as well as phosphorylation of eIF2α and activation of the IKKβ/NF-κB inflammatory pathway in the hypothalamus of mice and monkeys. In TNFα receptor 1 knockout mice (TNFR1−/−), AβOs failed to trigger hypothalamic IKK phosphorylation and IRS-1 inhibition. I.c.v.-injected oligomers induced peripheral glucose intolerance and hallmarks of insulin resistance, including adipose tissue inflammation and impaired insulin-induced surface translocation of GLUT-4 in skeletal muscle. The impact of AβOs in the hypothalamus preceded alterations in peripheral glucose homeostasis. AβO-associated glucose intolerance was prevented in TNFR1−/− mice and in wild type mice given i.c.v. infusions of tauroursodeoxycholic acid (TUDCA), an ER stress inhibitor. Collectively, results establish a novel pathogenic mechanism by which AβOs impact the hypothalamus and cause peripheral metabolic deregulation.

**Results**

Several studies have established that AβOs bind to hippocampal neurons and induce synapse loss and neuronal dysfunction, eventually leading to memory impairment in AD (e.g., (10;35-38). We initially aimed to determine whether AβOs also target hypothalamic neurons. To this end, AβOs were freshly prepared before each experiment and were routinely characterized by size exclusion chromatography, western blots using anti-oligomer monoclonal antibody NU4 (39) and, occasionally, by transmission electron microscopy, as previously described (40-42). Highly differentiated primary hypothalamic neuronal cultures were exposed to AβOs (500 nM) for 4 hrs and AβO binding to neurons was investigated by double immunofluorescence labeling using
oligomer-sensitive antibody NU4 (39) and microtubule-associated protein 2 (MAP-2). Results showed that AβOs bind to the soma and, especially, to dendrites of hypothalamic neurons (Figure 1A). We further asked whether AβOs would instigate oxidative stress in hypothalamic neurons, as previously shown for hippocampal neurons (43). We found that AβOs induce a robust increase in reactive oxygen species (ROS) levels in cultured hypothalamic neurons (Figure 1B).

To determine the impact of AβOs in an animal model with greater proximity to humans, we have recently developed a non-human primate model of AD by delivering i.c.v. infusions of oligomers in adult cynomolgus monkeys (Macaca fascicularis) (6;34). Three monkeys received i.c.v. injections of AβOs, while three sham-operated animals were used as controls, and their hypothalami were analyzed (Supplemental Figure 1). Strong AβO immunoreactivity was found in the hypothalamus of oligomer-injected monkeys (Figure 1C).

We next performed a single injection of 10 pmol AβOs into the lateral cerebral ventricle of male Swiss mice, an experimental model we have recently introduced to investigate in vivo hippocampal dysfunction and memory impairment in AD (44;45) (Supplemental Figure 2). Because ER stress was recently shown to underlie AβO toxicity in the hippocampus (34;46;47), and hypothalamic ER stress has been proposed to play an important role in the pathogenesis of metabolic disorders (48;49), we asked whether i.c.v.-infused AβOs induced ER stress in the mouse hypothalamus. We found a significant increase in hypothalamic levels of phosphorylated-eIF2α (eIF2α-P) 4 hours after i.c.v. injection of AβOs (Figure 2A), but not 7 days after oligomer injection (Figure 2B).

In animal models of T2D and obesity, an inflammatory response in the hypothalamus, notably via activation of the IKKβ/NFκB pathway, is an important part of the mechanism underlying pathogenesis (27;28). Compared to vehicle-injected mice, AβO-injected mice exhibited early activation of IKKβ in the hypothalamus (Figure 2C, 4 hours after i.c.v. injection), which persisted for 7 days after i.c.v. injection of AβOs (Figure 2D). Once activated, IKKβ phosphorylates IκBα, which undergoes ubiquitination and proteasomal degradation, allowing NFκB phosphorylation and migration to the nucleus. Accordingly, we found decreased levels of
IκBα (Figure 2E), a trend of increase in cytoplasmic NFκB phosphorylation (Figure 2F), and significantly increased levels of NFκB in the nucleus (Figure 2G) in the hypothalamus of AβO-injected mice. On the other hand, no differences in activated JNK or PKR levels were detected in the hypothalamus of AβO-injected mice compared with vehicle-injected mice 4 hours or 7 days after i.c.v. injection of oligomers (Supplemental Figure 3).

We further found that IRS-1pSer\(^{636}\) levels were increased and IRS-1pTyr\(^{465}\) levels were decreased in the hypothalamus of mice 7 days after oligomer injection (Figure 2H and I), indicating that AβOs induce impairment in hypothalamic insulin signaling. To determine whether AβOs caused insulin resistance in the hypothalamus, thereby impairing its ability to respond to insulin by reducing food intake, mice were kept in metabolic cages for seven days following i.c.v. injection of AβOs or vehicle and acute food intake following i.c.v. insulin infusion was measured (50;51). Significantly, AβO-injected mice showed no reduction in acute food intake upon i.c.v. administration of insulin, indicating hypothalamic insulin resistance (Figure 2J).

We next investigated whether similar effects to those found in mice could be observed in AβO-injected monkeys. We found significantly elevated hypothalamic levels of eIF2α-P (Figure 2K) and pIKKβ (Figure 2L), as well as a trend of decrease in hypothalamic IκBα levels in AβO-injected monkeys (Figure 2M). Results indicate that abnormal inflammatory signaling and ER stress are triggered by AβOs in the primate hypothalamus.

Intriguingly, AβO-injected mice presented increased chow intake (Figure 3A), even though no significant differences in body weight (Figure 3B) were found between experimental groups. Consistent with increased chow ingestion, elevated hypothalamic expression of orexigenic neuropeptides AgRP and NPY (but no alterations in anorexigenic POMC mRNA levels) were detected in AβO-injected mice (Figure 3C-E). Plasma levels of cholesterol and triglycerides were comparable between vehicle- and AβO-injected animals (Figure 3F and G).

Transient hypothalamic ER stress has been linked to up-regulation of the peripheral sympathetic tonus (48). We further found that AβO-induced hypothalamic inflammation and ER stress were
accompanied by elevated plasma noradrenaline (NA) levels (Figure 3H), indicating that AβOs cause deregulation of peripheral sympathetic control.

Collectively, the results described above led us to investigate whether centrally injected Aβ oligomers were capable of causing T2D-like peripheral metabolic deregulation in mice. We found that mice that received a single i.c.v. injection of 10 pmol AβOs exhibited impaired peripheral glucose tolerance and insulin resistance 7 days after injection (Figure 4A and B). Control experiments showed that peripheral glucose tolerance was unaffected by i.c.v. injection of a preparation of scrambled Aβ peptide submitted to the same oligomerization protocol used for regular Aβ preparations (Supplemental Figure 4A). Interestingly, the impairment in glucose tolerance induced by AβOs was comparable to that observed in mice submitted to a high-fat diet for 7 days (Supplemental Figure 4B). Impaired glucose tolerance could be detected as early as 36 hours, but not 12 hours after i.c.v. injection of AβOs (Supplemental Figure 4C and D), and persisted for at least 14 days after injection (Supplemental Figure 4E). We further examined the possibility that leakage of AβOs from the brain might explain the observed effects on peripheral glucose metabolism. To this end, we injected 10 pmol AβOs (the same amount used in i.c.v. injections) directly into the caudal vein or into the peritoneum of mice. In both cases, systemic administration of AβOs failed to impair glucose tolerance (Figure 4C and D), ruling out a direct action of AβOs on peripheral tissues in our conditions.

We next sought to analyze insulin-responsiveness in metabolically active tissues. We found increased CD68 immunoreactivity in adipose tissue of mice that received an i.c.v. injection of AβOs (Figure 5A), indicating macrophage/myeloid cell infiltration. Further, AβO-injected mice had higher amounts of epididymal fat (Figure 5B) and increased expression of leptin and pro-inflammatory cytokines, TNFα and IL-6, in white adipose tissue (Figure 5C-E). In obese mice, adipose tissue-derived TNFα is involved in insulin resistance through activation of JNK, leading to increased inhibitory serine phosphorylation of insulin receptor substrate-1 (IRS-1pSer) in muscle (52;53). Therefore, we investigated whether this pathway was affected in AβO-injected mice. Indeed, skeletal muscle from mice injected i.c.v. with AβOs showed increased levels of activated JNK (Figure 5F) and IRS-1pSer312 (Figure 5G). Physiologically, insulin signaling in muscle induces translocation of glucose transporter 4 (GLUT-4) from intracellular compartments
to the plasma membrane (54). In line with our finding of IRS-1 inhibition, insulin-stimulated translocation of GLUT-4 to the plasma membrane was severely impaired in skeletal muscle of mice that received an i.c.v. injection of AβOs (Figure 5H).

We recently reported that AβO-induced TNFα signaling in the hippocampus mediates impaired neuronal insulin signaling, synapse deterioration and memory loss in mice receiving i.c.v. infusions of AβOs (6;34). In addition, pioneering studies have established that activation of proinflammatory TNFα is a key mechanism leading to peripheral insulin resistance in diabetes (52;55;56), and that inhibition of hypothalamic inflammation prevents peripheral insulin resistance (57). Thus, we next investigated the effects of i.c.v.-injected AβOs in TNFα receptor 1 knockout (TNFR1−/−) mice. Interestingly, AβOs failed to induce glucose intolerance in TNFR1−/− mice (Figure 6A). TNFα signaling has been shown to activate intracellular stress kinases, including IKKβ (52;58). To determine whether TNFα signaling played a role in AβO-induced activation of hypothalamic IKKβ and IRS-1 inhibition in vivo, we injected AβOs i.c.v. in TNFR1−/− mice. Significantly, AβOs triggered IKKβ activation and IRS-1pSer636 in the hypothalamus of WT mice, but failed to do so in TNFR1−/− mice (Figure 6B and C).

Finally, recent observations indicate that transient hypothalamic ER stress is sufficient to deregulate peripheral insulin signaling and upregulate peripheral sympathetic tonus (48). Since we found that AβOs induce transient hypothalamic ER stress (Figure 2A and B) and increased plasma noradrenaline levels in mice (Figure 3H), we next investigated whether prevention of brain ER stress could attenuate AβO-induced defects in peripheral glucose homeostasis and in plasma noradrenaline levels. We found that i.c.v. injections of tauroursodeoxycholic acid (TUDCA), a chemical chaperone that alleviates ER stress, prevented both the impairment in glucose tolerance and the increase in plasma noradrenaline levels induced by i.c.v.-injected AβOs (Figure 6D and E).

Discussion

Diabetes and AD are chronic degenerative diseases increasing in prevalence in aging populations worldwide. Although clinical and epidemiological studies have linked AD to diabetes, with each disease increasing the risk of developing the other, the mechanisms of pathogenesis connecting...
them at the molecular and cellular levels remain to be elucidated. In particular, why AD patients present increased probability of developing diabetes is unknown. Here, we show that AβOs, toxins that accumulate in the AD brain and have been linked to neuronal dysfunction in brain areas related to learning and memory, impact the hypothalamus of mice and monkeys. Intriguingly, infusion of AβOs in the brain triggers peripheral insulin resistance in mice. These results provide initial evidence on the biological mechanisms underlying the clinical observations linking AD to diabetes.

Numerous studies have investigated the impact of AβOs in memory centers, specially the hippocampus (10), known to be fundamentally involved in the acquisition, consolidation, and recollection of new memories. This is because AD is classically recognized as a devastating disease of memory, and indeed memory-related brain regions have long been known to be affected in the course of disease (38;59). However, early studies indicated that other brain regions, not necessarily involved in learning and memory, may also be affected in AD. For example, postmortem analysis of AD brains identified Aβ deposits in the hypothalamus (30;31), and evidence of peripheral glucose intolerance in AD patients has been reported (20). More recently, voxel-based morphometry analysis showed reduced hypothalamic volume and a decreased number of orexin neurons in AD patients compared to healthy controls (32;33). Furthermore, hyperglycemia and hyperinsulinemia were shown to positively correlate with development of AD pathology (21). In transgenic mouse models of AD, obesity-induced insulin resistance is exacerbated (17;22). Collectively, these observations raise the intriguing possibility that the hypothalamus and peripheral glucose metabolism may be affected in AD. However, studies investigating the mechanisms underlying such clinical and postmortem observations are lacking. Using different experimental models, including cell-based assays, mice and monkeys that received i.c.v. injections of AβOs, we now show that functional alterations in the hypothalamus are induced by oligomers and lead to deregulated peripheral glucose handling.

In mice and monkeys, i.c.v. infusion of AβOs induced hypothalamic inflammation and endoplasmic reticulum stress, recently implicated as important pathogenic events in the onset of peripheral insulin resistance in metabolic disorders (25-28). While in mice we found a transient increase in hypothalamic eIF2α-P levels following a single i.c.v. injection of oligomers,
persistently elevated eIF2α-P levels were found in monkeys 3 days after the last in a series of injections of AβOs, suggesting that persistently elevated oligomer levels in the brain may induce prolonged effects in eIF2α-P. AβOs further induced IRS-1 inhibition (IRS-1pSer) in the hypothalamus of mice. It is noteworthy that oligomers failed to trigger hypothalamic IKKβ activation and IRS-1pSer in TNFR1−/− mice. Our results thus establish that AβO-induced TNFα/pIKK deregulation is directly linked to disrupted insulin signaling in the hypothalamus.

We have recently demonstrated that i.c.v.-injected AβOs disrupt insulin signaling and induce inflammation in the hippocampus of mice and monkeys (6;34;45). AβO-induced abnormal hippocampal TNFα signaling was found to be directly linked to synapse deterioration and cognitive impairment (34). It is thus possible that, in the AD brain, progressive accumulation of Aβ oligomers (due to elevated Aβ production or reduced clearance) brings about different functional outcomes in different brain regions. While the impact of AβOs in the hippocampus involves inflammation, ER stress and synapse deterioration, leading to memory deficits, AβO-induced inflammation and ER stress in the hypothalamus may be specially relevant in terms of disrupting hypothalamic insulin signaling. The hypothalamus is well-known for its ability to respond to changes in circulating insulin levels by regulating food ingestion (50). We found that an i.c.v. injection of insulin failed to repress short-term food ingestion in AβO-injected mice, suggesting that AβOs rendered the hypothalamus resistant to insulin. Remarkably, activation of a hypothalamic inflammatory pathway similar to the pathway we report in our model has been implicated as a central mechanism regulating energy imbalance in obese mice, and its suppression has been proposed to represent a potential strategy to combat obesity-related diseases (28). These findings further indicate that AβO- and obesity-induced hypothalamic inflammation share common pathogenic pathways.

We showed that no alterations in peripheral glucose homeostasis were detected 12 hours after an i.c.v. injection of AβOs (Supplemental Figure 4C), but markers of hypothalamic inflammation were already found to be elevated 4 hours after AβO infusion. This supports the notion that hypothalamic inflammation precedes and leads to peripheral metabolic alterations, a possibility that deserves further investigation. In this regard, an interesting recent study reported that, unlike inflammation in peripheral tissues, which develops as a consequence of obesity, hypothalamic
inflammatory signaling is evident in rats and mice prior to substantial weight gain and within 1 to 3 days of feeding on a high-fat diet (27), implicating hypothalamic inflammation in obesity pathogenesis (60). We further note that AβO-induced alterations in peripheral glucose homeostasis are similar to the alterations induced by a short period (7 days) of high-fat diet.

Our results establish that brain accumulation of AβOs affects the hypothalamus and impacts peripheral metabolism by mechanisms similar to those underlying peripheral insulin resistance in type 2 diabetes and other metabolic diseases. Similar to what has been described in metabolic disorders (60;61), i.c.v.-injected AβOs induce adipose tissue inflammation and impaired insulin-induced surface translocation of GLUT-4 in muscle cells. A previous work has reported that a high concentration of Aβ (10 μM) can induce hepatic insulin resistance in vitro through a direct effect on hepatocytes (18). However, in our model, AβOs failed to cause alterations in peripheral glucose homeostasis when delivered via the caudal vein or by injection into the peritoneum of mice, ruling out a direct effect of AβOs on peripheral tissues.

Activated JNK and PKR were recently implicated in AβO-induced defective hippocampal insulin signaling (6). However, these particular stress kinases do not appear to be involved in impaired hypothalamic insulin signaling, as no differences in pJNK and pPKR were detected in the hypothalamus of AβO-injected mice compared to vehicle-injected mice. This suggests the existence of region-specific mechanisms by which AβOs impair insulin signaling in the central nervous system.

Transient hypothalamic ER stress has been shown to induce increased levels of noradrenaline in the plasma of mice, and these effects have been reported as sufficient to induce peripheral glucose intolerance in mice (48). Consistent with this interesting study, here we showed that prevention of brain ER stress by administration of TUDCA prevented AβO-induced peripheral glucose intolerance and, further, normalized plasma noradrenaline levels. Attenuation of brain inflammation by infliximab, a TNFα-neutralizing antibody, further prevented alterations in peripheral glucose homeostasis. These results importantly demonstrate that hypothalamic ER stress and inflammation underlie alterations in peripheral glucose homeostasis induced by AβOs,
and suggest that pharmacological or other approaches to prevent hypothalamic dysfunction may provide novel therapeutics for metabolic deregulation in AD.

In conclusion, our findings establish that i.c.v.-injected AβOs trigger hypothalamic inflammation and cause peripheral glucose intolerance and insulin resistance. Results further support the emerging notion that pathological hypothalamic inflammation/ER stress leads to impaired peripheral glucose homeostasis. We propose the impact of AβOs on the hypothalamus comprises a key novel pathological mechanism that ultimately may disrupt peripheral metabolic homeostasis and lead to insulin resistance, revealing an important crosstalk between central and peripheral pathogenic mechanisms in AD. Our discovery that AβOs instigate hypothalamic deregulation draws attention to a brain structure that has been largely ignored to date in the study of AD pathogenesis, and highlights the importance of recognizing AD as a disease of both the brain and the periphery. As peripheral insulin resistance has been implicated in the development of AD (1;19), current results suggest the existence of a vicious cycle, instigated by brain accumulation of AβOs, contributing to the development of both AD and metabolic disorders, including type 2 diabetes.

Materials and Methods

Preparation of Aβ oligomers. Oligomers were prepared from synthetic Aβ1-42 peptide (American Peptide, Sunnyvale, CA) as originally described (36). The peptide was initially solubilized in hexafluoroisopropanol (HFIP) and the solvent was evaporated to produce dried films, which were subsequently dissolved in sterile anhydrous dimethylsulfoxide to make a 5 mM solution. This solution was diluted to 100 μM in ice-cold PBS and incubated overnight at 4 °C. The preparation was centrifuged at 14,000x g for 10 min at 4 °C to remove insoluble aggregates (protofibrils and fibrils), and the supernatants containing soluble Aβ oligomers were stored at 4 °C. Protein concentration was determined using the BCA assay (Pierce, Deerfield, IL). Routine characterization of oligomer preparations was performed by size-exclusion chromatography and Western blotting using anti-Aβ 6E10 (Abcam, Cambridge, MA) or anti-Aβ oligomer NU1 (39)
monoclonal antibodies, and, occasionally, by transmission electron microscopy, as previously described (41;42;44). Oligomers were used within 48 hours of preparation.

*Mature hypothalamic neuronal cultures, immunocytochemistry and ROS assays.* Primary hypothalamic neuronal cultures were prepared from rat embryos (E18) according to procedures established for hippocampal neuronal cultures (e.g., (13;43)). Cultures were plated at a density of 70,000 cells/cm² on poly-L-lysine-coated coverslips and were maintained for 18-21 days *in vitro* in Neurobasal medium with B27 supplement and L-glutamine (0.5 mM). For experiments designed to determine reactive oxygen species (ROS) formation, 20,000 cells/cm² were plated directly on poly-L-lysine-coated wells of 96-well plates. After 18-21 days *in vitro*, cultures were incubated for 4 h at 37 °C with vehicle or 500 nM AβOs. ROS formation was assessed using 2 μM of the fluorescent probe CM-H₂DCFDA (Invitrogen, Carlsbad, CA), as previously described (43). CM-H₂DCFDA is sensitive to the formation of various types of ROS, including peroxide, hydroxyl radical, peroxyl radicals, and peroxynitrite. After 30 min of loading with the fluorescent probe, neurons were rinsed three times with warm PBS and two times with neurobasal medium without phenol red. Cells were immediately imaged on a Nikon Eclipse TE 300-U fluorescence microscope. At least three experiments with independent neuronal cultures were performed, each with triplicate well per experimental condition. Three images were acquired from randomly selected fields per well. Results obtained in independent experiments were combined to allow quantitative estimates of changes in neuronal ROS levels. Quantitative analysis of immunofluorescence data was carried using Image J (Windows Version) using appropriate thresholding to eliminate background signal before histogram analysis, as described (43).

Immunocytochemistry was performed as previously described (13). Briefly, hypothalamic cultures were treated for 3 h at 37 °C with 500 nM AβOs or equivalent volumes of vehicle, and were fixed for 10 minutes with 4% paraformaldehyde containing 4% sucrose in PBS. Cells were blocked for 1 hour with 10% normal goat serum in PBS and incubated with monoclonal AβO-selective NU4 antibody (1:2,000; (39)) for 16 hours. Neurons were rinsed 3 times with PBS, permeabilized with 0.1% Triton-X100 for 5 minutes and incubated for two hours at room temperature with anti-MAP2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA; 1:200).
Neurons were rinsed 3 times with PBS and incubated for two hours at room temperature with Alexa Fluor555 anti-mouse IgG and Alexa Fluor488 anti-rabbit IgG (1:2,000). After washing, cells were mounted on coverslips using Prolong Gold Antifade with DAPI (Invitrogen), and were imaged on a Zeiss Axio Observer Z1 Microscope equipped with an Apotome module.

Animals and intracerebroventricular (i.c.v.) injections. Male Swiss mice obtained from our own animal facility were 2.5-3 month-old at the beginning of experiments. TNFR1−/− female mice in a C57/BL6 background and wild-type littermates were obtained from the University of Campinas Breeding Centre (CEMIB). Animals were housed in groups of five in each cage with free access to food and water, under a 12h light/dark cycle, with controlled room temperature and humidity. All procedures used in the present study followed the “Principles of Laboratory Animal Care” (US National Institutes of Health) and were approved by the Institutional Animal Care and Use Committee of the Federal University of Rio de Janeiro (protocol IBqM 072-05/16). For i.c.v. injection of AβOs, animals were anesthetized for 7 min with 2.5% isoflurane (Cristália, São Paulo, Brazil) using a vaporizer system (Norwell, MA) and were gently restrained only during the injection procedure itself, as recently described (44). A 2.5 mm-long needle was unilaterally inserted 1 mm to the right of the midline point equidistant from each eye and 1 mm posterior to a line drawn through the anterior base of the eye (44;62;63); see Fig. S1). Ten pmol of AβOs (concentration expressed in terms of Aβ monomers) or vehicle were injected in 30 seconds, in a total volume of 3 μL for Swiss mice and 1 μL for C57/BL6 mice. Injection of 3 μL of a blue dye into the lateral ventricle of Swiss mice was performed to verify diffusion along the CSF circulation so as to reach the whole brain (Supplemental Figure 2). At the end of experiments, injection of blue dye in the same injection site used for AβOs or vehicle was employed to verify the accuracy of injection into the lateral ventricle. Mice showing any signs of misplaced injections or brain hemorrhage (~ 5% of animals throughout our study) were excluded from further analysis.

In experiments using monkeys, 6 cynomolgus monkeys (Macaca fascicularis; body weights 4.7-7.0 kg) were used. Monkeys were maintained at the Centre for Neuroscience at Queen’s University (Kingston, Canada) under the close supervision of a lab animal technician and the Institute veterinarian. All animals had a cannula implanted in the lateral ventricle by aseptic surgery. Anesthesia was induced by ketamine (10 mg/kg, intramuscular). During surgery,
glycopyrrolate (0.013 mg/kg) and isoflurane (1-3%) were also used. Correct placement of the cannula was assessed by MRI. After a recovery period, three monkeys received intracerebroventricular injections of 100 μg of AβOs (one injection per day every 3 days for 24 days). Three sham-operated monkeys were used as controls. Upon completion of the experimental protocol, monkeys were sedated with intramuscular ketamine (10 mg/kg) plus buprenorphine (0.01 mg/kg) for analgesia, followed by intravenous sodium pentobarbital (25 mg/kg), perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde in PBS; 4% paraformaldehyde in PBS containing 2.5% glycerol; PBS + 5% glycerol; and PBS + 10% glycerol. All procedures were approved by the Queen’s University Animal Care Committee and were in full compliance with the Canada Council on Animal Care (Animal Care Protocol Original Munoz-2011-039-Or).

**Immunohistochemistry in monkey brain sections.** Immunohistochemistry was performed using free floating serial 40 μm-thick coronal sections in PBS containing 1% Triton incubated with 0.1 M citrate buffer, pH 6, at 60 °C for 5 minutes. Endogenous peroxidase was inactivated by incubation of sections with 3% hydrogen peroxide in methanol for 2 hours. Sections were then blocked with 5% bovine serum albumin (BSA) and 5% normal goat serum (NGS) in 1% Triton X-100 for 3 hours at room temperature. Primary antibodies against phospho-eIF2α (Enzo Life Sciences; 1:200), phospho-IKKβ (Abcam; 1:200) and IκBα (Cell Signaling; 1:200) were diluted in blocking solution and sections were incubated at 4 °C for 16 hours, followed by incubation with biotinylated secondary antibody for 2 hours at room temperature, and then processed using the Vectastain Elite ABC reagent (Vector Laboratories) according to manufacturer's instructions. The sections were washed in PBS and developed using DAB in chromogen solution, and counterstained with Harris's hematoxylin. Slides were mounted with Entellan (Merck) and imaged on a Zeiss Axio Observer Z1 microscope. Omission of primary antibody was routinely used to certify absence of nonspecific labeling (data not shown). For immunofluorescence analysis, tissue autofluorescence was quenched by incubation with 0.06% potassium permanganate for 10 min at room temperature. Sections were blocked in 5% bovine serum albumin (BSA) and 5% normal goat serum (NGS) in 1% Triton X-100 for 3 hours at room temperature. Primary antibody against AβOs (NU4; (39); 1:300) was diluted in blocking solution and sections were incubated at 4 °C for 16 hours, followed by incubation with Alexa555-
conjugated anti-mouse secondary antibody (1:1,500) for 2 hours at room temperature. Slides were mounted with Prolong Gold Antifade with DAPI (Invitrogen) and imaged on a Zeiss Axio Observer Z1 microscope equipped with an Apotome module to minimize out-of-focus light.

**Immunohistochemistry in mouse tissues.** For GLUT-4 immunohistochemistry, mice received one i.c.v. injection of vehicle or 10 pmol AβOs. Seven days later, mice received one i.p. injection of either PBS or insulin (1 IU/kg body weight) and were killed by decapitation 15 minutes later. The soleus muscle was dissected and fixed in 4% paraformaldehyde. After 48 hours, tissues were cryoprotected in sucrose (20-30%) and 20 μm sections were obtained in a cryostat (Leica CM1850). Sections were fixed with acetone for 30 min, washed twice with PBS and incubated for 1 hour with rabbit polyclonal anti-Glut4 antibody (Abcam; 1:500). Sections were then incubated with Alexa555-conjugated anti-rabbit antibody (1:1000; Invitrogen) for 1 hour and mounted in Prolong Gold Antifade with DAPI (Invitrogen). Sections were imaged on a Zeiss Axio Observer Z1 microscope equipped with an Apotome module. Eight images were acquired per section and integrated immunofluorescence intensity was determined using Image J Software (Windows Version). For adipose tissue immunohistochemistry, mice injected i.c.v. with vehicle or AβOs were killed seven days after injection and samples of epididymal adipose tissue were removed and fixed in 4% paraformaldehyde. After 48 hours, tissues were included into paraffin blocks, and 3 μm sections were obtained using a microtome and mounted in slides. For immunohistochemistry, slides were immersed in xylene for 10 minutes, sequentially rehydrated in absolute, 95% and 70% ethanol in water, and incubated with 3% H2O2 in methanol for inactivation of endogenous peroxidase. Antigens were reactivated by treatment with 0.01 M citrate buffer for 40 min at 95 °C. Slides were washed in PBS and incubated with CD68 antibody (Abcam; 1:200) for 12-16 hours at 2-8 °C. After washing with PBS, slides were incubated with biotinylated secondary antibody for 1 hour, washed twice with PBS and incubated with streptavidin-biotin-peroxidase for 30 min. Slides were then covered with 3,3’ diaminobenzidine solution (0.06% DAB in PBS containing 2% DMSO and 0.018% H2O2) for 1 to 5 min or until a brown precipitate could be observed. Identical conditions and reaction times were used for slides from different animals to allow comparison between immunoreactivity densities. Reaction was stopped by immersion of slides in distilled water. Counter-staining was performed with Harris hematoxilin. Four images were randomly acquired for each animal using a Zeiss Axio Observer.
Z1 microscope. An optical density threshold that best discriminated staining from background was obtained using NIH Image J 1.36b imaging software (NIH, Bethesda, MD).

**Intraperitoneal Glucose Tolerance Test (GTT).** Mice were fasted for 12 hours and blood samples were collected from a tail incision. After collection of a baseline sample, mice received an i.p. injection of glucose (2 g/kg body weight). Blood glucose measurements were repeated at 15, 30, 45, 60 and 120 minutes after glucose injection, using a One-Touch® Ultra® Glucose Meter and strips (Johnson & Johnson).

**Intraperitoneal Insulin Tolerance Test (ITT).** Mice were fasted for 5 hours and blood samples were collected from a tail incision. After collection of a baseline sample, mice received an i.p. injection of insulin (1 IU/kg body weight). Blood glucose measurements were repeated at 15, 30, 45 and 60 minutes after insulin injection, using a One-Touch® Ultra® Glucose Meter and strips (Johnson & Johnson). If blood glucose levels fell below 20 mg/dL, mice were immediately given an i.p. injection of glucose and were excluded from the experiment. Kitt was calculated as described (64).

**High-fat diet.** Mice were maintained for seven days on normal chosoow or a high-fat diet containing 55% of energy derived from fat, 29% from carbohydrates and 16% from protein, prepared as described (64;65).

**Treatment with tauroursodeoxycholic acid (TUDCA).** Mice received 5 µg TUDCA i.c.v. per injection. Injections were carried out 20 minutes prior to AβO injection, and at 2, 24 and 96 hours thereafter. An extra TUDCA injection was given 12 hours before the GTT, which was performed seven days after AβO administration. Twenty four hours after the GTT, mice were deeply anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine, and blood samples were collected by cardiac puncture in heparinized tubes. Plasma was separated by centrifugation at 3,000x g at 4 °C for 10 min and samples were used for noradrenaline quantification (as described below).
**Intracaudal injections.** Animals were anesthetized with halothane and aseptically injected via the tail vein with 10 pmol AβOs or Dulbecco’s PBS, in a final injection volume of 100 μl.

**Determination of accumulated food intake and intracerebroventricular insulin injection.** Swiss mice were submitted to stereotaxic surgery for implantation of a cannula directed to the third ventricle, as described (64). Mice were allowed to recover from surgery in their home cages for four days before being placed in individual metabolic cages. Animals then received i.c.v. injections of vehicle or AβOs, and food intake was measured every day at the same time for seven days. Mice then received an i.c.v. injection of PBS or insulin (200 mU) at the beginning of the dark cycle, and food intake was determined by the difference between chow given to mice immediately after injection and the weight of remaining chow 12 hours after.

**Noradrenaline extraction and quantification.** Norepinephrine levels in plasma were measured by HPLC separation coupled with electrochemical detection (HPLC-ED). Perchloric acid was added to plasma samples to a final concentration of 0.1 M. Samples were centrifuged (10,000x g) to remove precipitated proteins and supernatants were used for automated injection into the HPLC. Fast isocratic separation was obtained using a reverse phase LC-18 column (4.6 mm x 250 mm; Supelco) with the following mobile phase: 20 mM sodium dibasic phosphate, 20 mM citric acid, pH 2.64, containing 10% methanol, 0.12 mM Na₂EDTA, and 566 mg/L heptanesulphonic acid.

**Western blots.** Four hours, six hours or seven days after i.c.v. injection of AβOs (as indicated in “Results”), mice were euthanized by decapitation and the hypothalamus and gastrocnemius muscle were rapidly dissected and frozen in liquid nitrogen. For total protein extraction, samples were thawed and homogenized in buffer containing 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP-40 (Invitrogen), 1% sodium deoxycholate, 0.1% SDS, 5 mM EDTA, 1% Triton X-100 and phosphatase and protease inhibitor cocktail (Pierce–Thermo Scientific, Rockford, IL). Protein concentration was determined using the BCA kit. Aliquots containing 30 μg protein were resolved by SDS-PAGE in 4-20% polyacrylamide gels (Invitrogen) and were electrotransferred to nitrocellulose or PVDF membranes for one hour at 300 mA. Blots were blocked for one hour with 5% non-fat dry milk in Tween-Tris buffer solution at room temperature or with Odyssey Blocking Buffer (LI-COR, Lincoln, NE; 1:2 dilution in Tween-Tris Buffer), and were incubated
overnight at 2 °C with primary antibodies diluted in blocking buffer. Primary antibodies used were IRS-1pSer^{636} (Santa Cruz; 1:200), IRS-1pSer^{312} (Invitrogen; 1:200), IRS-1pTyr^{465} (Santa Cruz; 1:200), total IRS-1 (Santa Cruz; 1:200), pJNK (Thr^{183}/Tyr^{185}) monoclonal antibody (Cell Signaling; 1:1,000), JNK polyclonal antibody (Cell Signaling; 1:1,000), phospho-eIF2α (Enzo Life Sciences; 1:1,000), total eIF2α (Abcam; 1:1,000), pIKKβ (Abcam; 1:1,000), total IKKβ (Abcam; 1:1,000), IκBα (Cell Signaling; 1:1,000), pNFκB p65 (Ser^{536}; Cell Signaling; 1:1,000), total NFκB p65 (Santa Cruz; 1:250), p-PKR (Santa Cruz; 1:200), total PKR (Santa Cruz; 1:250), β-tubulin III (Sigma-Aldrich, St. Louis, MO; 1:10,000) and β-actin (Cell Signaling; 1:10,000). After overnight incubation with primary antibodies, membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:30-50,000), IRDye800CW- or IRDye680RD-conjugated secondary antibodies (LI-COR; 1:10,000) at room temperature for two hours. Chemiluminescence was developed using SuperSignal West Femto (Thermo Fisher Scientific). Alternatively, fluorescence intensities were quantified in an Odyssey CLx apparatus (LI-COR).

**Nuclear enriched fractions.** For preparation of nuclear extracts, hypothalamus of vehicle- or AβO-injected mice were homogenized in 0.1 mL hypotonic lysis buffer (10 mM Hepes, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM dithiothreitol plus a phosphatase and protease inhibitor cocktail) for 15 min at 4 °C. Cells were then lysed by adding 0.5% Nonidet P-40. The homogenate was centrifuged (13,000x g for 5 min at 4 °C), and supernatants containing the cytoplasmic extracts were stored at −80 °C. The nuclear pellet was resuspended in 75 μL ice-cold hypertonic extraction buffer (20 mM Hepes, pH 7.9, 300 mM NaCl, 1.5 mM MgCl₂, 0.25 mM EDTA, 25% glycerol, 0.5 mM dithiothreitol plus phosphatase and protease inhibitors). After 40 min of intermittent mixing, extracts were centrifuged (13,000x g for 20 min at 4 °C), and supernatants containing nuclear proteins were saved. Total protein concentration was determined using the BCA kit. Aliquots containing 20 μg protein were resolved by SDS-PAGE in 4-20% polyacrylamide gels (Invitrogen) and were electrotransferred to nitrocellulose membranes for one hour at 300 mA. Blots were processed and incubated with antibodies as described above.

**RNA extraction and quantitative Real-Time PCR analysis.** Hypothalamus and adipose tissue from vehicle- or AβO-injected mice were homogenized in 500 or 1000 μl Trizol (Invitrogen),
respectively, and RNA extraction was performed according to manufacturer’s instructions. Purity and integrity of RNA were determined by the 260/280 nm absorbance ratio and by agarose gel electrophoresis. Only preparations with ratios > 1.8 and no signs of RNA degradation were used. In adipose tissue samples, a 30 min long incubation at 30 °C was performed, the lipid layer was removed and discarded, and RNA extraction was performed in the water soluble phase. One μg RNA was used for cDNA synthesis using the SuperStrand III Reverse Transcriptase kit (Invitrogen). Expression of genes of interest was analyzed by qPCR on an Applied Biosystems 7500 RT-PCR system using the Power SYBR kit (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. Primer pairs used are shown in Table 1. Cycle threshold (Ct) values were used to calculate fold changes in gene expression using the $2^{-\Delta Ct}$ method. In all cases, reactions were performed in 15 μl reaction volumes.

**Table 1. Primer sequences used for qPCR reactions**

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPY</td>
<td>ATG CTA GGT AAC AAG CGA ATG G</td>
<td>TGT CGC AGA GCG GAG TAG TAT</td>
</tr>
<tr>
<td>POMC</td>
<td>ATG CCG AGA TTC TGC TAC AGT</td>
<td>TCC AGC GAG AGG TCG AGT TT</td>
</tr>
<tr>
<td>AgRP</td>
<td>ATG CTG ACT GCA ATG TTG CTG</td>
<td>CAG ACT TAG ACC TGG GAA CTC T</td>
</tr>
<tr>
<td>IL6</td>
<td>TTC TTG GGA CTG ATG CTG GTG</td>
<td>CAG AAT TGC CAT TGC ACA ACT C</td>
</tr>
<tr>
<td>TNFα</td>
<td>CCC TCA CAC TCA GAT CAT CTT CT</td>
<td>GCT ACG ACG TGG GCT ACA G</td>
</tr>
<tr>
<td>Leptin</td>
<td>TGA GCT ATC TGC AGC ACG TT</td>
<td>TTC ACA CAC GCA GTC GGT AT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>AGG TCG GTG TGA TGA ACG GAT TTG</td>
<td>TGT AGA CCA TGT AGT TGA GGT CA</td>
</tr>
</tbody>
</table>
Reference List


defective thermogenesis, insulin resistance, and impaired insulin secretion. 

_Endocrinology_ 152:1314-1326.


Acknowledgements: This work was supported by grants from Human Frontiers Science Program (HFSP) (to FGF), National Institute for Translational Neuroscience (INNT/Brazil) (to STF), the Brazilian funding agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (to STF, FGF, CPF and JRC), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (to STF, FGF, CPF and JRC), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Canadian Institutes for Health Research (CIHR) and Canada Research Chair Program (to DPM). We thank Drs. Matthias Gralle and Jordano Brito-Moreira for performing AβO injections in monkeys, Drs. Brito-Moreira and Adriano Sebollela for chromatographic characterization of oligomer preparations, Dr. Leonardo M. Saraiva for help with hypothalamic neuronal cultures and ROS assays, Maíra S. Oliveira, Mariângela M. Viana, Sandra Bambrilla and Dioze Guadagnini for technical support, and Ana Claudia Rangel for secretarial and accounting support. WLK is co-founder of Acumen Pharmaceuticals, which has been licensed by Northwestern University to develop ADDL technology for Alzheimer's therapeutics and diagnostics.
Figure 1. AβOs bind to hypothalamic neurons and trigger ROS formation. A, Representative immunocytochemistry images of mature hypothalamic neurons in culture exposed to vehicle (Veh) or AβOs (500 nM) for 3 hours. Binding of AβOs was detected using anti-oligomer monoclonal antibody NU4 (red). Neurons were double-labeled using anti-MAP-2 antibody (green). Nuclear staining (DAPI) is shown in blue. Images represent typical results from experiments with 3 independent hypothalamic cultures (3 coverslips/experimental condition used in each experiment). Scale bar = 20 μm. B, Representative DCF fluorescence images from hypothalamic neuronal cultures exposed to vehicle or AβOs (500 nM, 4 hours). The inset show optical zoom of the indicated area. Scale bars = 100 μm and 50 μm for main panels and insets, respectively. Graph shows integrated DCF fluorescence intensities (relative units; see Methods) (n = 3 independent hypothalamic cultures; 3 wells/experimental condition in each experiment; 3 images acquired per well). C, Representative images of AβO-immunoreactivity
(detected using anti-oligomer monoclonal antibody NU4) in the hypothalamus of control, sham-operated adult cynomolgus monkeys (Sh; n = 3) or monkeys that received i.c.v. injections of AβOs (n = 3; see Methods). Nuclear staining (DAPI) is shown in blue. Insets show optical zoom images of the areas indicated by white dashed rectangles in the main panels. Scale bars = 100 and 20 μm for main panels and insets, respectively. Bars represent means ± S.E.M. # p = 0.08 (one-sample t-test compared with a fixed value of 100 RU$s$).
Figure 2. AβOs induce hypothalamic inflammation and ER stress. A and B, Western blot analysis of eIF2α-P levels in the hypothalamus of mice 4 hours (A) or 7 days (B) after i.c.v. injection of vehicle (Veh) or AβOs. Graphs show densitometric data normalized by total eIF2α levels. C and D, Western blot analysis of hypothalamic phospho-IKKβ levels in the hypothalamus of mice 4 hours (C) or 7 days (D) after i.c.v. injection of vehicle or AβOs. Graphs show densitometric data normalized by total IKKβ levels. E and F, Western blot analysis of IκBα (E) and cytoplasmic phospho-p65-NF-κB (F) in the hypothalamus of mice 4 hours after i.c.v. injection of vehicle or AβOs. Graphs show densitometric data normalized by actin (E) or...
total NF-κB levels (F). G, Nuclear NF-κB was measured in the hypothalamus 6 hours after i.c.v. injection of vehicle or AβOs in mice. Graphs show NF-κB levels normalized by nuclear marker, lamin. H and I, IRS-1pSer$^{636}$ and pTyr$^{465}$ were measured in the hypothalamus 7 days after i.c.v. injection of vehicle or AβOs in mice. Graphs show IRS-1p levels normalized by total IRS-1. (n = 4-7/group for all Western blot experiments using Swiss mice). J, Twelve-hour food intake after a single i.c.v. infusion of insulin (200 mU) in mice 7 days after i.c.v. injection of vehicle or AβOs (n = 6-10 animals/group). K-M, Representative images showing eIF2α-P (K), phospho-IKKβ (L) and IκBα (M) immunoreactivities in the hypothalamus of cynomolgus monkeys that received i.c.v. injections of AβOs or control (sham-operated; Sh) monkeys (n = 3 animals/group). Insets show optical zoom images of areas indicated by white dashed rectangles in the main panels. Scale bars = 50 μm. Graphs show immunolabeling optical density analysis (see Methods) from 3 images acquired in the hypothalamus of each monkey (3 control versus 3 AβO-injected animals). Data are expressed as means ± S.E.M. A-I: * p<0.05; ** p<0.01, Student’s t-test. J: *** p<0.001, one-way ANOVA followed by Bonferroni post hoc test comparing Veh-Insulin versus PBS groups. K-M: * p<0.05 and # p = 0.05, Student’s t-test.
Figure 3. I.c.v.-injected AβOs induce increased food intake, hypothalamic expression of orexigenic neuropeptides and increased plasma levels of noradrenaline. A, Accumulated chow intake (normalized by body weight) measured during 7 days following i.c.v. injection of vehicle or AβOs in mice (n = 7-12 animals/group). B, Daily body weight measured during 7 days after i.c.v. injection of vehicle or AβOs (n=10 animals/group). C-E, Adult Swiss mice (n = 4-6 animals/group) received a single i.c.v. injection of vehicle or 10 pmol AβOs and hypothalamic levels of mRNA for AgRP (C), NPY (D) and POMC (E) were analyzed 7 days after injection. F-H, Plasma levels of total cholesterol (F), triglycerides (G) and noradrenaline (H) (n=6-8 animals/group) measured 7 days after i.c.v. injection of vehicle or AβOs. Data are expressed as means ± S.E.M; * p<0.05; *** p<0.001, Student’s t-test.
Figure 4. I.c.v.-injected AβOs induce impaired peripheral glucose tolerance and insulin resistance. A, Adult Swiss mice (n = 9-16 animals/group) received a single i.c.v. injection of vehicle or 10 pmol AβOs and were assessed in a glucose tolerance test (2 g glucose/kg body weight, i.p.) 7 days after injection. Blood levels of glucose were measured at several time points following glucose administration. Bar graph represents areas under the curves in the scatter plot. B, Insulin tolerance test (1 IU insulin/kg body weight, i.p.) (n = 9-16 animals/group). Blood levels of glucose were measured at several time points following insulin administration. Bar graph represents the kinetic constants for glucose disappearance (Kitt) calculated from the scatter plot. C and D, Glucose tolerance test (2 g glucose/kg body weight, i.p.) in mice 7 days after a single intracaudal (C; n = 8 animals/group) or intraperitoneal (D; n = 13 animals/group) injection of AβOs (10 pmol) or vehicle. Data are expressed as means ± S.E.M. A and B, left panels: * p<0.05; ** p<0.01 and *** p<0.001, two-way ANOVA. A and B, right panels: * p<0.05; ** p<0.01, Student’s t-test.
Figure 5. I.c.v-injected AβOs induce adipose tissue inflammation and insulin resistance in muscle. A, CD68-immunoreactivity in white adipose tissue (scale bar = 25 μm) and B, epididymal fat content were analyzed in mice (n = 6 animals/group) 7 days after i.c.v. injection of vehicle or AβOs. C-E, Relative expression of leptin, TNFα and IL-6, respectively, in white adipose tissue of mice (n = 7-9 animals/group) 7 days after i.c.v injection of vehicle or AβOs. F and G, p-JNK and IRS-1pSer312 levels (normalized by total JNK and GAPDH, respectively) in the muscle of mice (n = 4 animals/group) 7 days after i.c.v injection of vehicle or AβOs. H, Representative images of GLUT-4 immunofluorescence in insulin-stimulated muscle from mice that were i.c.v.-injected with vehicle (Veh) or 10 pmol AβOs. Bar graphs show quantification of GLUT-4 surface immunoreactivity in skeletal muscle of mice that received intraperitoneal injections of PBS or insulin (1 IU/kg body weight) 7 days after i.c.v. injection of vehicle or AβOs, as indicated (n = 5 animals/group). Scale bar = 25 μm. Data are expressed as means ±
S.E.M. A-G. * p<0.05, Student’s t-test. H, * p<0.05, two-way ANOVA followed by Bonferroni post-hoc Veh-Insulin versus AβO-Insulin.
Figure 6. AβO-induced peripheral glucose intolerance and hypothalamic insulin resistance are mediated by TNFα signaling and hypothalamic ER-stress. A, Glucose tolerance test (2 g glucose /kg body weight, i.p.) in TNFR1−/− mice or wild type littermates performed 7 days after i.c.v. injection of vehicle or AβOs. Bar graph represents areas under the curves (AUC) in the scatter plot. B and C, Western blot analysis of phospho-IKKβ (B) and IRS-1pSer636 levels (C) in the hypothalamus of wild-type (WT) or TNFR1−/− mice 10 days after i.c.v. injection of vehicle or AβOs (n = 5 animals/experimental group). D, Glucose tolerance test (2 g glucose /kg body weight, i.p.) in mice i.c.v.-injected with vehicle, vehicle + TUDCA, AβOs or AβOs + TUDCA (when used, TUDCA was administered in 5 i.c.v. injections of 5.0 μg TUDCA each; see Methods). Glucose tolerance test (GTT) was performed 7 days after i.c.v. injection of vehicle or AβOs. Bar graph represents areas under the curves (AUC) in the scatter plot. E, Plasma noradrenaline (NA) levels measured 7 days after i.c.v. injection of vehicle, vehicle + TUDCA, AβOs or AβOs + TUDCA in mice (n = 8-12 animals/group). Representative images from Western blot experiments were always run on the same gels but represent noncontiguous lanes. Data are expressed as mean ± S.E.M; In A and D left panels: ** p<0.01 and *** p<0.001, two-way ANOVA. A and D right panels and B-D: * p < 0.05, one-way ANOVA followed by Bonferroni post-hoc, compared to vehicle-injected mice.