INSULIN RESISTANCE AND METABOLIC FAILURE UNDERLIE ALZHEIMER DISEASE

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Abstract: Alzheimer disease (AD) is the most common cause of dementia in North America. Despite 30+ years of intensive research, gaps remain in our understanding of AD pathogenesis and approaches to treatment. However, the recent rapid shift to a paradigm that focuses on the roles of metabolic dysfunction and insulin and insulin-like growth factor (IGF) resistance as causal agents of cognitive impairment and neurodegeneration holds promise. The overarching hypothesis, that AD is a brain diabetes (type 3), accounts for the impairments in neuronal survival, myelin maintenance, energy metabolism, synaptic integrity, and plasticity, and the well-recognized neuropathological processes including, tau hyper-phosphorylation, amyloid-beta (A\textsubscript{\textbeta}-\textbeta) accumulation, oxidative and endoplasmic reticulum stress, and cerebral microvascular disease. Herein, we discuss the roles of aging, lifestyle choices, peripheral insulin resistance diseases, including obesity, type 2 diabetes mellitus, nonalcoholic fatty liver disease, and metabolic syndrome, nitrosamine exposures, and familial/genetic factors as mediators of brain diabetes, cognitive impairment, and neurodegeneration. The data suggest that neurodegeneration can be initiated and propagated by the buildup of agents consequential to peripheral insulin resistance, i.e. toxic lipids (ceramides), and predicts that toxic ceramides generated in liver or visceral fat, cross the blood-brain barrier and cause brain insulin resistance, stress, and inflammation. This extrinsic mechanism of neurodegeneration accounts for the strikingly concurrent and overlapping increases in prevalence of all insulin resistance diseases. Yet, there is evidence that AD/type 3 diabetes occurs as the dominant or only manifestation of insulin resistance. The predicted intrinsic pathway of neurodegeneration is nearly identical to the extrinsic pathway, except its underlying basis is direct toxic/metabolic injury to the brain, or familial AD-associated mutations and gene variants that accelerate the trajectory to brain insulin resistance with aging. Finally, we propose that progressive cognitive impairment and neurodegeneration in AD are effectuated by a positive feedback mal-signaling cascade, whereby declining function of insulin/IGF networks dysregulate lipid metabolism and increase local levels of toxic ceramides. Toxic ceramides promote inflammation, endoplasmic reticulum and oxidative stress, and mitochondrial dysfunction, all of which exacerbate brain insulin/IGF resistance. Over time and with aging, adducts accumulate in DNA, RNA, protein, and lipids, causing continuous multi-modal molecular failure, leading to disruption of cytoskeletal function, A\textalpha\textbetaPP-A\textbeta secretion, synaptic plasticity, cell survival mechanisms, and myelin maintenance. Once established, the reverberating loop must be targeted using multi-pronged approaches to disrupt spiraling progression of the AD neurodegeneration cascade.
1.1 INTRODUCTION

The mature brain requires intact insulin and insulin-like growth factor (IGF) signaling for homeostasis, neuronal plasticity, and myelin integrity. Resistance and deficiency of insulin and IGF disrupt energy balances and signaling networks that are needed to support a broad range of functions, including cell survival. In recent years, considerable evidence has accumulated showing that in Alzheimer disease (AD), cognitive impairment and neurodegeneration are associated with insulin and IGF resistance and impairments in signaling through pro-growth and pro-survival pathways. Furthermore, studies have linked the sharply increased incidence and prevalence rates of AD to other chronic insulin resistance disease states, including obesity, type 2 diabetes mellitus, nonalcoholic fatty liver disease, and metabolic syndrome. On the other hand, there is ample evidence that sporadic AD very frequently occurs in the absence of peripheral insulin resistance diseases. In addition, because familial forms of AD, although relatively uncommon, have nearly identical clinical and neuropathological features as sporadic AD, their disease mechanisms ultimately should be shared with those of sporadic AD. This review focuses on two major questions: (1) how do peripheral insulin resistance diseases contribute to the pathogenesis of cognitive impairment and neurodegeneration; and (2) do the same pathogenic factors mediate AD neurodegeneration, whether or not peripheral insulin resistance diseases or mutations in the amyloid precursor protein or presenilin genes exist?

These concepts share in common the theme of insulin resistance with dysregulated lipid metabolism. Consequences include increased local tissue and peripheral blood levels of cytotoxic ceramides. Cytotoxic ceramides promote inflammation, oxidative stress, endoplasmic reticulum (ER) stress, and worsened insulin resistance. We propose that peripheral insulin resistance diseases promote or exacerbate cognitive impairment and neurodegeneration by causing brain insulin resistance. Mechanistically, toxic ceramides generated in liver or visceral fat leak into the peripheral circulation due to local cellular injury or death. The lipid-soluble nature of the toxic ceramides enables them to cross the blood-brain barrier, and either initiate or propagate a cascade of neurodegeneration mediated by brain insulin resistance, inflammation, stress, and cell death (extrinsic pathway).

Human and experimental data indicate that sporadic AD occurs in the absence of diabetes, obesity, fatty liver disease, or metabolic syndrome. Yet, AD brains exhibit significant deficits in insulin and IGF signaling, which worsen as the disease progresses. Clues pertaining to the pathogenesis of disease stem from epidemiological and experimental studies. Epidemiological studies strongly support exposure rather than genetic factors as agents of disease. Experimental models highlight the role of nitrosamine and related toxins as mediators of insulin resistance diseases, including in the brain. Mechanistically, we propose that the nitrosamines (toxins) present in processed and preserved foods, cause oxidative damage, disrupt lipid metabolism, and impair insulin signaling. Toxic lipids (ceramides) accumulated directly in the brain promote inflammation, stress, and insulin resistance, which together activate a positive feedback mal-signaling cascade that causes AD-type neurodegeneration (intrinsic pathway). Familial forms of AD are discussed in light of how their gene mutations (AβPP, PS1, and PS2) or variants (ApoE-ε4) prematurely disrupt brain insulin/IGF signaling networks, and thereby accelerate brain aging. These concepts help delineate the strategies needed to detect, monitor, treat, and prevent AD, as well as other major insulin resistance diseases.

1.2 MEDIATORS OF INSULIN SIGNALING

1.2.1 Insulin, the Master Hormone

Insulin is a 5800 Dalton, 51 amino acid polypeptide, composed of an A (21 residues) chain and B (30 residues) chain linked by disulfide bonds. In the early 1920s, Banting and Best discovered insulin in pancreatic secretions [1, 2], and shortly thereafter demonstrated that it reversed hyperglycemia in humans [3]. It took nearly 30 years to devise methods that could stabilize insulin, prolong its actions, and delay its absorption, and it took 50 years to produce 99% pure insulin, free of pro-insulin and other islet polypeptides [4]. Prior to 1980, insulin used to treat humans was extracted from bovine or porcine
pancreas, but early in 1980, commercial sources of human insulin became available.

Advancement of molecular and biochemical technology, including genetic engineering, led to large-scale production of human insulin. Currently, human insulin is produced using Saccharomyces cerevisiae (yeast) technology, and insulin is continuously harvested from the supernatant during fermentation. Insulin is further purified, crystalized, esterified, and hydrolyzed to ensure purity [5]. However, efforts are currently underway to produce insulin that can be administered orally rather than by injection [6].

1.2.2 Insulin-Stimulated Effects

The main targets of insulin include skeletal muscle, adipose tissue, and liver, although virtually every organ, tissue, and cell type is responsive to insulin stimulation. Insulin regulates glucose uptake and utilization by cells and regulates free fatty acid levels in peripheral blood. Free fatty acids are substrates for generating complex lipids. In skeletal muscle, insulin stimulates glucose uptake by inducing translocation of the glucose transporter protein, GLUT4, from the Golgi to the plasma membrane [7]. In liver, insulin stimulates lipogenesis and triglyceride storage, and inhibits gluconeogenesis. In adipose tissue, insulin decreases lipolysis and fatty acid efflux [8]. These pro-metabolic effects of insulin on glucose and free fatty acid disposal help to maintain energy balance.

1.2.3 Insulin-Like Growth Factors (IGFs)

Insulin is closely related to another polypeptide, insulin-like growth factor 1 (IGF-1). IGF-1 is also referred to as somatomedin C or mechano growth factor [9, 10]. IGF-1 regulates growth, particularly during development, and it exerts anabolic effects on mature organs and tissues. IGF-1 is composed of 70 amino acids (7649 Daltons) in a single chain that contains three intra-molecular disulfide bridges [9, 10]. IGF-1 is abundantly produced in liver, and its supply and actions are regulated by interactions with IGF binding proteins (IGFBPs) [11].

1.2.4 Insulin and IGF Signaling in the Brain

Historically, most of the research concerning insulin and IGF actions focused on cells and tissues other than those of the central nervous system (CNS). However, within the last 15 to 20 years, information has steadily emerged about expression and function of insulin and IGF polypeptides and receptors in the CNS. In the brain, insulin and IGF signaling regulates a broad array of neuronal and glial activities, including growth, survival, metabolism, gene expression, protein synthesis, cytoskeletal assembly, synapse formation, neurotransmitter function, and plasticity [12, 13]. In addition, insulin and IGF pathways have critical roles in maintaining cognitive function. Insulin, IGF-1, and IGF-2 polypeptide and receptor genes are expressed in neurons [12] and glial cells [14, 15] throughout the brain, but their highest levels of expression are in structures that are typically targeted by neurodegenerative diseases [12, 16, 17]. The fact that genes encoding insulin, IGFs, and insulin-like peptides and their receptors are expressed in human, rodent, and drosophila brains [18] suggests that the corresponding signaling networks permit local control of diverse functions, including energy metabolism.

1.2.5 Insulin and IGF Signal Transduction: Steps in Pathway Activation

The mechanisms of insulin/IGF signaling in the brain are the same as those in non-CNS cells. Insulin and IGF networks are activated by binding of trophic factors to their own receptors, which promotes phosphorylation and activation of intrinsic receptor tyrosine kinases. Subsequent interactions between the tyrosine phosphorylated receptors and insulin receptor substrate (IRS) proteins mediate downstream transmission of signals. Positive signaling to inhibit apoptosis and to stimulate growth, survival, metabolism, and plasticity is effected by activating phosphoinositol-3-kinase (PI3K)-Akt and extracellular mitogen-activated protein kinase (Erk MAPK), and by inhibiting glycogen synthase kinase 3β (GSK-3β) [17].

1.2.6 Cross Talk between Insulin/IGF and Other Major Signal Transduction Networks in the Brain

Insulin/IGF-1 pathways cross talk with other major networks, including Wnt/β-catenin and Notch [19,
20]. Wnt/β-catenin and Notch also support diverse neuronal functions; disruption of these pathways has been implicated in the pathogenesis of neurodegeneration [21, 22]. Wnt/β-catenin signaling regulates neuronal proliferation, migration, differentiation, axon outgrowth, and synaptic plasticity [23–26]. In contrast, GSK-3β inhibits Wnt by phosphorylating β-catenin. This event destabilizes the Axin/APC/β-catenin complex and targets β-catenin for ubiquitin/proteasome-mediated degradation [20, 27–30]. Notch signaling promotes cell adhesion and remodeling in the CNS, and is activated by insulin/IGF [31]. Given the breadth of functions supported by insulin/IGF signaling, including its cross talk with Wnt/β-catenin and Notch, one would logically conclude that any significant impairment in insulin and IGF signaling would have dire consequences on the structural and functional integrity of the CNS.

1.3 INSULIN RESISTANCE AND NEURODEGENERATION

1.3.1 Insulin Resistance and Its Consequences

Insulin resistance is classically defined as the state in which high levels of blood insulin (hyperinsulinemia) are associated with hyperglycemia. The concept has broadened to include organ and tissue-related impairments in insulin signaling associated with reduced activation of the pathways. As a result, progressively higher levels of ligand are needed to achieve normal insulin actions [7]. However, sustained high levels of insulin can also cause insulin resistance [32], thereby worsening and possibly broadening tissue involvement. Furthermore, hyperinsulinemia impairs insulin secretion from β-cells in pancreatic islets, yielding hybrid states of both insulin resistance and insulin deficiency [32].

Long-term consequences of insulin resistance include cellular energy failure (lack of fuel), elevated plasma lipids, and hypertension. In addition, chronic hyperinsulinemia vis-à-vis normoglycemia predicts future development of diabetes mellitus [33]. Insulin resistance is an independent predictor of serious diseases including cerebrovascular and cardiovascular disease, hypertension, and malignancy [34–38]. Insulin resistance is now front and center stage because of its link to our seemingly unbridled obesity, type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD), metabolic syndrome, polycystic ovarian disease, age-related macular degeneration, and Alzheimer disease (AD) epidemics.

1.3.2 Alzheimer Disease (AD)

AD Occurrence and Clinical Diagnosis AD is the most common cause of dementia in North America. Sporadic AD, which has no clear pattern of genetic transmission, accounts for more than 90% of the cases, whereas familial (heritable) forms of AD account for 5–10% of all cases. Throughout the past several decades, sporadic AD has become epidemic, raising questions about environmental and lifestyle mediators of cognitive impairment and neurodegeneration [39]. Although the clinical diagnosis of AD is based on criteria set by the National Institute of Neurological and Communicative Disorders and Stroke, the Alzheimer Disease and Related Disorders Association (NINCDS/ADRDA), and DSM-IV criteria [40], embracement of additional tools such as neuroimaging and standardized biomarker panels have helped facilitate detection of the early brain metabolic derangements in AD [41].

AD Neuropathology Neuropathological hallmarks of AD include: neuronal loss; abundant accumulations of abnormal, hyper-phosphorylated cytoskeletal proteins in neuronal perikarya and dystrophic fibers; and increased expression and abnormal processing of amyloid-beta precursor protein (AβPP), leading to AβPP-Aβ peptide deposition in neurons, plaques, and vessels. The gold standard for definitively diagnosing AD is to demonstrate beyond-normal aging associated densities of neurofibrillary tangles, neuritic plaques, and AβPP-Aβ deposits in corticolimbic structures, bearing in mind that neurodegeneration frequently involves multiple other cortical regions. The common thread among these lesions is that they harbor insoluble aggregates of abnormally phosphorylated and ubiquitinated tau, and neurotoxic AβPP-Aβ in the form of oligomers, fibrillar aggregates, and plaques. Secreted neurotoxic AβPP-Aβ oligomers inhibit hippocampal long-term
potentiation, i.e. synaptic plasticity [42], which is needed for learning and memory.

1.3.3 Concept: AD is a Metabolic Disease Driven by Brain Insulin/IGF Resistance

For more than 30 years, research efforts have been squarely focused on the pathogenic roles of hyperphosphorylated tau and AβPP-Αβ in AD. However, growing evidence is highlighting the importance of insulin resistance and metabolic dysfunction as mediators of AD [43,44]. In essence, AD could be regarded as a metabolic disease tied to and possibly caused by brain insulin and IGF resistance [45,46]. Correspondingly, AD shares many features in common with systemic (non-CNS) insulin resistance diseases. For example, reduced insulin-stimulated growth and survival signaling, increased oxidative stress, proinflammatory cytokine activation, mitochondrial dysfunction, and impaired energy metabolism occur in both AD and peripheral insulin resistance diseases [17,47,48]. Even in its early stages, AD is marked by deficits in cerebral glucose utilization [49–51]. As AD progresses, brain metabolic derangements [52,53] and impairments in brain insulin signaling, insulin-responsive gene expression, glucose utilization, and metabolism worsen [45,46,54].

1.3.4 Brain Insulin and IGF Resistance and Deficiency in AD-Human Studies

Human postmortem studies established that brain insulin resistance mediated by reduced insulin receptor expression and insulin receptor binding were consistent and fundamental abnormalities in AD [45,46,54]. Moreover, impairments in signaling are not restricted to insulin pathways as they also involve IGF-1 and IGF-2 networks [45,46]. AD-associated deficits in brain insulin and IGF signaling progress with disease severity [45] and involve pathways needed to maintain neuronal survival, energy production, gene expression, and plasticity [43]. Correspondingly, nearly all the critical features of AD could logically represent consequences of brain insulin/IGF resistance. These features include increases in (1) activation of kinases that aberrantly phosphorylate tau and lead to accumulation of neurofibrillary tangles, dystrophic neuritic plaques, and neuropil threads; (2) expression of AβPP and accumulation of AβPP-Αβ peptides that are neurotoxic and result in senile plaque formation; (3) oxidative and ER stresses, which propagate cell death cascades; (4) mitochondrial dysfunction that causes energy deficits; and (5) disruption of cholinergic homeostasis needed for neuronal plasticity, memory, and cognition.

1.3.5 AD = Type 3 Diabetes

Unlike systemic forms of diabetes, AD-associated deficits in insulin/IGF signaling are due to the combined effects of insulin/IGF resistance and deficiency. Insulin/IGF resistance is manifested by reduced levels of insulin/IGF receptor binding and decreased responsiveness to insulin/IGF stimulation, whereas the trophic factor deficiency is associated with reduced levels of insulin polypeptide and gene expression in brain and cerebrospinal fluid [44–46]. In essence, AD can be regarded as brain diabetes that has elements of both insulin resistance (T2DM) and insulin deficiency (T1DM). To consolidate this concept, we proposed that AD be referred to as “type 3 diabetes” [45,46].

1.3.6 Experimental Type 3 Diabetes (Sporadic AD)

The hypothesis that AD is actually a brain form of diabetes is supported by experimental data showing that intracerebroventricular injection of streptozotocin, a pro-diabetes drug, causes rats to develop deficits in spatial learning and memory, along with brain insulin resistance, brain insulin deficiency, and AD-type neurodegeneration, but not diabetes mellitus [55,56]. On the other hand, intraperitoneal or intravenous administration of streptozotocin causes diabetes mellitus with relatively mild hepatic steatosis and neurodegeneration [57,58]. Therefore, brain diabetes (type 3) can occur independent of type 1 or type 2 diabetes, and vice versa.

Further studies utilized small interfering RNA duplex molecules to silence the expression of insulin and IGF receptors in the brain [59] without causing genotoxic and nitrosative damage, which occur with streptozotocin [57]. The results showed that disruption of brain insulin and IGF receptors was
sufficient to cause cognitive impairment and hippocampal degeneration with molecular abnormalities similar to those in AD [59]. However, it was also evident that the oxidative and nitrosative damage were needed to produce a more robust model. Finally, human and experimental studies demonstrated neuroprotective effects of glucagon-like peptide-1 (GLP-1) [60], IGF-1 [61], and caloric restriction [62], which respectively stimulate insulin actions, slow brain aging, and reduce insulin resistance. Together, these studies support the notion that AD is a brain diabetes-type metabolic disease mediated by local insulin and IGF resistance.

1.4 THE NEUROPATHOLOGY OF AD IS CAUSED BY BRAIN INSULIN/IGF RESISTANCE

1.4.1 Overview
Chronic insulin/IGF-1 resistance has dire consequences on the functional integrity of the brain [12, 63] due to impairments in neuronal survival, energy production, gene expression, and plasticity [43]. Inhibition of insulin/IGF signaling contributes to AD by increasing: (1) the activity of kinases that aberrantly phosphorylate tau and therefore compromise neuronal cytoskeletal integrity; (2) the expression of AβPP and accumulation of AβPP-αβ; (3) oxidative stress; (4) endoplasmic reticulum (ER) stress; and (5) metabolic dysfunction with attendant activation of pro-inflammatory and pro-death cascades. Functional consequences of brain insulin/IGF resistance include down-regulation of target genes needed for cholinergic homeostasis, and compromise of systems that mediate neuronal plasticity, memory, and cognition [43–46].

1.4.2 Tau Pathology
Neurofibrillary tangles, dystrophic neurites, and neuropil threads are major neuronal cytoskeletal lesions that correlate with dementia in AD [64]. At the core of these lesions are aggregates of hyperphosphorylated, ubiquitinated, insoluble fibrillar tau. Tau, a neuronal microtubule-associated protein, becomes hyperphosphorylated due to inappropriate activation of kinases such as GSK-3β [65], cyclin-dependent kinase 5 (cdk-5), and c-Abl [66], and/or inhibition of protein phosphatases 1 and 2A [66, 67]. Hyperphosphorylation causes tau to misfold, self-aggregate, and form insoluble fibrils (paired helical filaments and straight filaments) [68] that eventually develop into neurofibrillary tangles, dystrophic neurites, and neuropil threads [67]. Intra-neuronal accumulations of fibrillar tau disrupt neuronal cytoskeletal structure and function, impairing axonal transport and synaptic integrity. Synaptic disconnection is one of the hallmarks of AD neurodegeneration [67]. In addition, pre-fibrillar tau can aggregate into neurotoxic soluble oligomers or insoluble granular deposits that promote disconnection of synapses and death of neurons [69]. Ubiquitination of hyper-phosphorylated tau [70], together with eventual dysfunction of the ubiquitin-proteasome system [71], worsen the accumulation of insoluble fibrillar tau. Fibrillar tau exerts its neurotoxic effects by increasing oxidative stress, ROS generation, neuronal apoptosis, mitochondrial dysfunction, and necrosis [72].

Several aspects of the molecular and structural pathology of tau in AD are explainable on the basis of brain insulin/IGF resistance [17, 45, 46, 73, 74]. First, tau gene expression and phosphorylation are regulated by insulin and IGF [63]. Impairments in insulin/IGF signaling contribute to tau hyper-phosphorylation due to over-activation of specific kinases, e.g. GSK-3β and Cdk-5, and impaired tau gene expression [17, 75]. Consequences include failure to generate sufficient quantities of normal soluble tau protein, vis-à-vis accumulation of hyperphosphorylated insoluble fibrillar tau, with attendant cytoskeletal collapse, neurite retraction, and synaptic disconnection. Second, decreased signaling through phosphoinositol-3-kinase (PI3K), Akt [63], and Wnt/β-catenin [76], and increased activation of GSK-3β [65] correlate with brain insulin and IGF resistance. Impairments in signaling through these pathways could account for the reductions in neuronal survival, myelin maintenance, synaptic integrity, neuronal plasticity, mitochondrial function, and cellular stress management in AD.

1.4.3 Amyloid-Beta (AβPP-Aβ)
AD is marked by dysregulated expression and processing of amyloid precursor protein (AβPP), with
attendant accumulation of AβPP-AB neurotoxic oligomeric fibrils or insoluble larger fibrillar aggregates (plaques). Mechanistically, increased AβPP expression and altered proteolysis lead to accumulation of 40 or 42 amino acid AβPP-AB peptides that aggregate. In familial/inherited forms of AD, mutations in the AβPP, presenilin 1 (PS1), and PS2 genes, or inheritance of the Apolipoprotein E ε4 (ApoE-ε4) allele, are responsible for increased synthesis and deposition of AβPP-AB peptides in the brain [77,78]. In sporadic AD, which accounts for 90% or more of the cases, the cause of AβPP-AB accumulation is still debated. However, recent evidence suggests that impaired insulin/IGF signaling promotes AβPP-AB accumulation due to dysregulated AβPP expression and protein processing [74].

The concept that AβPP-AB toxicity causes insulin resistance, and the opposing argument, that brain insulin resistance with oxidative stress and neuro-inflammation promote AβPP-AB accumulation and toxicity, are both supported by experimental data. Studies have shown that insulin stimulation accelerates trafficking of AβPP-AB from the trans-Golgi network, where it is generated, to the plasma membrane, and that insulin stimulates AβPP-AB extracellular secretion [79] and inhibits its intracellular accumulation and degradation by insulin-degrading enzyme (IDE) [80]. On the other hand, in hyper-insulin states, IDE may become diverted to degrade excess insulin, leaving AβPP processing deficient and allowing AβPP-AB to accumulate [81]. Whether these actions actually contribute to AβPP-AB burden is not known. However, it is clear that impaired insulin signaling can disrupt processing of AβPP and clearance of AβPP-AB [82]. Accumulation of AβPP-AB exacerbates the problem because AβPP-AB disrupts insulin signaling by competing with insulin, or reducing the affinity of insulin for binding to its own receptor [83]. In addition, AβPP-AB oligomers inhibit neuronal transmission of insulin-stimulated signals by desensitizing and reducing surface expression of insulin receptors. Furthermore, intracellular AβPP-AB directly interferes with PI3 kinase activation of Akt, which leads to impaired survival signaling, increased activation of GSK-3β, and hyper-phosphorylation of tau. Because IGF-1 or IGF-2 suppression of GSK-3β [84] reduces the neurotoxic effects of AβPP [85], the neuro-protective properties of these and related trophic factors could be exploited for treatment of AD.

1.4.4 Oxidative Stress

Chronic insulin/IGF resistance increases both oxidative and endoplasmic reticulum (ER) stress [48]. High levels of persistent oxidative stress lead to formation of reactive oxygen (ROS) and reactive nitrogen (RNS) species, which are present in AD brains [86]. ROS and RNS are problematic because they attack subcellular organelles, including mitochondria, and thereby exacerbate oxidative stress. In addition, molecular attacks resulting in stable adducts formed with DNA, RNA, lipids, and proteins, compromise the structural and functional integrity of neurons [87]. Consequences include loss of plasma membrane and ER functions, disruption of the neuronal cytoskeleton with dystrophy and synaptic disconnection, deficits in neurotransmitter release and neuronal plasticity, and perturbations of cellular homeostasis and survival mechanisms.

Oxidation of amino acid residues results in formation of advanced glycation end products (AGEs) or advanced oxidation protein products. Oxidation causes proteins to unfold, and renders them inactive and susceptible to cleavage. Oxidation of aliphatic side-chains produces peroxides and carbonyls (aldehydes and ketone). Peroxide attack on other molecules generates radicals. Carbonyls are toxic and cause stress-induced AGE accumulation, which contributes to progressive loss of cellular functions in aging, diabetes, human AD, experimental AD, and other degenerative diseases [88,89]. Therefore, elevated levels of AGE in AβPP-AB plaques and neurofibrillary tangles [90–92] may contribute to progressive cell loss with neurodegeneration [87,90,92].

1.4.5 Endoplasmic Reticulum Stress

Endoplasmic reticulum (ER) functions, such as protein synthesis, modification, and folding, calcium signaling, and lipid biosynthesis, are driven by glucose metabolism. In insulin resistance states, such as in obesity, T2DM, NASH, and metabolic syndrome, impairments in glucose uptake and utilization are
associated with increased ER stress pathway activation [93–95]. Chronically high levels of ER stress lead to dysregulated lipid metabolism, accumulation of toxic lipids, e.g. ceramides, and activation of pro-inflammatory and pro-apoptosis cascades [96, 16, 97]. Recent studies showed that ER stress and dysregulated lipid metabolism occur in human brains with AD, and worsen with severity of disease and progression of brain insulin/IGF resistance [48].

### 1.4.6 Metabolic Deficits—The Starving Brain

Insulin and IGF signaling regulate glucose utilization and ATP production in the brain. In AD, deficits in cerebral glucose utilization and metabolism occur early and prior to significant cognitive decline [98]. Therefore, impairments in brain insulin signaling are probably pivotal to AD pathogenesis [46]. Supporting data were provided by experimental animal models in which brain insulin/IGF resistance was associated with cognitive impairment and AD-type neurodegeneration [55, 99]. Oxidative stress and ROS can damage mitochondrial membranes, making them more permeable, and mitochondrial DNA, impairing electron transport and ATP production and worsening ROS. Furthermore, oxidative stress and its responses can (1) activate pro-inflammatory networks that exacerbate organelle dysfunction and pro-apoptosis mechanisms; (2) stimulate AβPP gene expression [100] and AβPP cleavage, resulting in increased formation of AβPP-Aβ neurotoxic fibrils [85]; and (3) activate or dis-inhibit GSK-3β, which promotes tau phosphorylation. Therefore, oxidative stress stemming from brain insulin/IGF resistance and metabolic dysfunction contribute to neuronal loss, AβPP-Aβ toxicity, tau cytoskeletal pathology, and neuro-inflammation in AD [12, 45, 101].

Glucose uptake in the brain is mediated by the GLUT4 transporter [102], which is abundantly expressed along with insulin receptors, in the medial temporal lobe, as well as other notable targets of AD [12, 17]. Insulin stimulates GLUT4 mRNA, and GLUT4 protein trafficking from the Golgi to the plasma membrane where it engages in glucose uptake. In AD, although GLUT4 mRNA expression is preserved [46], deficits in brain glucose utilization and energy metabolism vis-à-vis brain insulin/IGF resistance could be mediated by functional impairments in GLUT4, i.e. post-translational mechanisms responsible for GLUT4 trafficking to the plasma membrane. Resulting deficiencies in energy metabolism increase oxidative stress [73] and help drive pro-apoptosis, pro-inflammatory, and pro-AβPP-Aβ cascades, which worsen DNA damage, mitochondrial dysfunction, oxidative stress, and ROS generation [12, 17, 45, 46, 55].

### 1.4.7 Chronic Ischemic Cerebral Microvascular Disease

Cerebral microvascular disease is a consistent feature of AD, and recognized mediator of cognitive impairment. Postmortem studies demonstrated similar degrees of dementia in people who had severe AD versus moderate AD plus chronic ischemic encephalopathy. The ischemic injury mainly consisted of multifocal small infarcts and leukoaraiosis, i.e. extensive white matter fiber attrition with pallor or myelin staining [103]. T2DM and hypertension are known causes of microvascular disease throughout the body, including the brain. Evidence that microvascular disease contributes to neurodegeneration was suggested by the finding of progressive medial temporal lobe atrophy with advancement of T2DM [104].

Hyperinsulinemia, as occurs with insulin resistance in T2DM, causes progressive injury to microvessels, ultimately producing a state of chronic cerebral hypoperfusion. Chronic microvascular injury is characterized by reactive proliferation of vascular endothelial cells, thickening of the intima, fibrosis of the media, and narrowing of the lumens. Mural scarring reduces vascular compliance and compromises blood flow and nutrient delivery, particularly in periods of high metabolic demand. Moreover, blood vessel walls are rendered leaky and therefore permeable to toxins due to their structural weakness [105,106]. These effects could account for the perivascular tissue attrition (widened perivascular spaces) seen in brains of people with T2DM. In AD, restricted blood flow and oxygen/nutrient delivery compounds the adverse effects of insulin/IGF resistance by further increasing oxidative stress, thereby activating signal transduction pathways that promote aberrant tau phosphorylation, AβPP...
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1.5 BRAIN METABOLIC DERANGEMENTS IN OTHER NEURODEGENERATIVE DISEASES

Advances in neuro-imaging techniques, including the use of positron emission tomography (PET) scanning, magnetic resonance imaging (MRI), functional MRI, and magnetic spectroscopy, combined with sophistication of molecular and biochemical analyses of postmortem human brains have helped to demonstrate common themes among different forms of neurodegeneration [107–109]. One such important finding was that besides AD, other important neurodegenerative diseases are associated with deficits in brain metabolism. Correspondingly, Parkinsonism-dementia with Lewy bodies (DLB), fronto-temporal lobar dementias, motor neuron disease, and multiple systems atrophy are also associated with accumulations of mis-folded ubiquitinated proteins (often cytoskeletal), oxidative stress, neuroinflammation, autophagy, mitochondrial dysfunction, apoptosis, and necrosis [110–114]. Furthermore, epidemiologic studies linked Parkinson disease to diabetes mellitus [60], and experimental 6-OHDA-induced PD to striatal insulin resistance [115].

As an example of the probable mechanistic interrelatedness of neurodegenerative disease, we demonstrated significant reductions in IGF-1 and IGF-2 receptor binding, and expression of IGF-2 polypeptide, and IGF-1 and IGF-2 receptors in the frontal lobes of patients with DLB [116]. DLB and AD differ because in DLB, the most prominent abnormalities are centered around IGF-1 and IGF-2 networks, whereas in AD, insulin signaling is most disrupted, followed by IGF-1 and IGF-2 activated pathways. In DLB, impairments in frontal lobe IGF-1 and IGF-2 signaling were associated with reductions in neuronal, neurotrophin, and tyrosine kinase neurotrophin receptor expression, and increased levels of α-synuclein, dopamine-β-hydroxylase, lipid peroxidation, and ubiquitin immunoreactivity [116]. Altogether, these findings suggest that more attention should be paid to brain metabolic dysfunction as a primary mediator of neurodegeneration.

1.6 UNDERLYING CAUSES OF BRAIN INSULIN RESISTANCE IN AD

1.6.1 Aging

Insulin and IGF resistance increase with aging in all organisms, whereas longevity is associated with preservation of insulin/IGF responsiveness [81, 117, 118]. There is some evidence that cumulative challenges and stresses over a lifespan damage cells and tissues due to excessive signaling through insulin/IGF-1 receptors. For example, mutation of the daf-2 gene, which encodes a hybrid insulin/IGF-1 receptor in C. elegans, extends lifespan [119]. In contrast, mutations that reduce insulin/IGF-1 signaling and caloric restriction, which reduce utilization of insulin networks, extend lifespan and decelerate degenerative aging. Correspondingly, neuronal over-expression of insulin receptor substrate 2 (IRS2) leads to increased fat mass, insulin resistance, and glucose intolerance with aging [120]. These findings lead to the conclusion that chronic overuse of insulin/IGF signaling networks, which occurs with hyperinsulinemia and insulin resistance, is deleterious because it fast-forwards the aging process.

Declines in growth hormone levels and metabolism also play potentiate aging due to the co-occurrence of anabolic deficiencies that accelerate metabolic dysfunction and mortality [121]. Because growth hormone deficiency promotes obesity [122], and obesity promotes insulin resistance and hyperinsulinemia, aging-associated declines in growth hormone could mediate their effects by causing insulin resistance [123]. Resulting impairments in energy balance increase oxidative stress, activate pro-inflammatory pathways, and encourage formation of ROS. The fallout includes progressive accumulation of mitochondrial DNA adducts, DNA damage, mitochondrial dysfunction, and finally cell death. Because this concept is broadly applicable to degenerative diseases that are linked to insulin resistance, improvements in our understanding of aging could help generate new measures to prevent or delay the onset of neurodegeneration.

Arguments could be made that insulin resistance, cognitive impairment, and AD are inevitable consequence of aging [124], and therefore, anyone who lives long enough will develop these diseases.
Mechanistically, the chronic low-grade inflammation associated with aging [125, 126] is correlated with insulin resistance [126, 127]. Inflammation and insulin resistance increase oxidative stress. Over time, ROS and AGEs form, driving mitochondrial dysfunction, DNA damage, and activation cell death cascades [123]. These factors certainly could account for aging-associated cognitive impairment and brain atrophy.

However, a formidable argument can be made that other critical factors dictate the consequences of aging because: (1) the rates and characteristics of aging vary widely among individuals; (2) the nature and target organs diseased by insulin resistance are heterogeneous; and (3) there is no clear reason why aging per se should result in chronic inflammation, insulin and IGF resistance, or growth hormone deficiency. To account for individual and possibly population-based variability in aging and insulin resistance, we hypothesize that host compensatory mechanisms that are ample in youth, become disabled by the consequences of aging. This process exposes underlying genetic or epigenetic templates that dictate host and organ-system susceptibility to insulin resistance. Genetic factors could be inheritance of FAD-associated genes. Epigenetic factors could be wear and tear effects of poor lifestyle choices, including diet. An excellent example of this phenomenon exists with respect to post-polio syndrome, in which people who recovered from childhood poliomyelitis develop motor neuron disease as middle-aged adults [128, 129]. Mechanistically, aging-associated loss of the compensatory scaffolding build by a youthful plastic CNS dramatically exposes the underlying previously damaged motor neuron system [130, 131]. In the previously undamaged CNS, aging simply results in mild weakness and reduced mobility. This scenario raises the question about the need to chronically enrich and protect neuronal circuitry to maintain excellent function throughout the normal human lifespan.

1.6.2 Lifestyle-Induced Accelerated Aging

Until about 40 or 50 years ago, aging and its consequences, including insulin resistance, seemed ordinary and inevitable. However, in relatively recent times, we have witnessed rapid increases in prevalence of insulin resistance-related diseases among non-aged individuals, including adolescents and children. In other words, type 2 diabetes, non-alcoholic fatty liver disease, metabolic syndrome, cognitive impairment, and cardiovascular diseases are occurring earlier [124] and at epidemic rates. These new trends are linked to progressive increases in the prevalence of obesity and the commonplace adaptation of sedentary lifestyles. Because the profiles of insulin resistance diseases in younger age groups are nearly identical to those associated with aging, arguments could be made that certain lifestyles, habits, and behaviors cause disease by accelerating aging. At the same time, this concept suggests that lifestyle modifications may retard aging and defer or altogether prevent aging-associated insulin resistance diseases, including neurodegeneration.

1.6.3 Peripheral Insulin Resistance Diseases

Overview Insulin resistance diseases, including AD, obesity, T2DM, NASH, and metabolic syndrome have grown in prevalence in virtually all cultures, but these global problems began in modern high technology societies, particularly the United States. These diseases, which are now epidemic [39, 132, 133], consume high percentages of healthcare budgets, lead to disability, and cause premature death. Major contributing factors include our unrelenting appetite for highly processed, starch-laden, fat-laden, calorically dense foods that are rendered “tasty” by commercial industries. Unfortunately, this seemingly addictive behavior is eroding health status across all age groups. Thanks to robust international research efforts, we now know that insulin resistance can cause chronic degenerative disease in virtually any organ due to dysregulated energy metabolism, and increased inflammation, oxidative and nitrosative stress, and proneness to cell death. Unfortunately, commercial luring continues to draw the uninformed to adopt the “conveniences” of Western lifestyles. Consequently, insulin resistance diseases are quickly spreading throughout the world and have begun to bear their toils on global health.

Obesity Obesity significantly increases risk for T2DM, NAFLD, NASH, metabolic syndrome, and cognitive impairment. Obesity caused by chronic
high caloric intake is sufficient to disrupt homeostatic mechanisms and cause insulin resistance [8, 134–136]. Concerns regarding obesity’s effects on the brain stemmed from epidemiological and clinical studies showing that people with glucose intolerance, deficits in insulin secretion, T2DM, obesity/dyslipidemic disorders, or NASH were at higher risk for developing mild cognitive impairment (MCI) or AD-type dementia [17, 137–139]. Further investigations demonstrated that obese individuals have impairments in executive function [139, 140], and their risks for developing AD is at least two-fold higher than in the general population [141]. Correspondingly, experimental animal models with diet-induced obesity and T2DM exhibit cognitive declines [62] with deficits in spatial learning and memory [142], and brain atrophy with insulin resistance, inflammation, oxidative stress, and cholinergic dysfunction [134, 143]. In humans, weight loss that is sufficient to reduce peripheral insulin resistance, improves cognitive performance [144, 145] and enhances neuropsychiatric function [146]. Similarly, adherence to a Mediterranean diet reduces metabolic indices and lowers the risk for AD [147].

**Type 2 Diabetes Mellitus (T2DM)** The molecular and biochemical abnormalities in brains with AD mimic the effects of T2DM or NASH on skeletal muscle, adipose tissue, and liver. Epidemiological studies showed that individuals with glucose intolerance, deficits in insulin secretion, or T2DM were at significantly increased risk for developing mild cognitive impairment (MCI) or AD-type dementia. Longitudinal studies revealed that diagnoses of T2DM [148] and obesity/dyslipidemic disorders [149] correlate with subsequent development of MCI, dementia, or AD [148, 150]. However, further postmortem studies demonstrated that, although peripheral insulin resistance states contribute to cognitive impairment and AD progression in humans, they did not independently cause AD [151, 152]. Similarly, in experimental models of chronic high-fat diet feeding and diet-induced obesity with T2DM, cognitive impairment with deficits in spatial learning and memory [142] were associated with mild brain atrophy, brain insulin resistance, neuro-inflammation, oxidative stress, and deficits in cholinergic function, but not overt AD [134, 153].

**Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Fatty Liver Disease (NAFLD)** The fact that obesity per se, may not be an independent risk factor for MCI and neurodegeneration suggests that specific effects pertaining to obese states govern these propensities [154]. In this regard, several studies have shown that cognitive impairment and neuropsychiatric dysfunction occur with liver disease caused by obesity, alcohol abuse, chronic Hepatitis C virus infection, Reyes syndrome, and nitrosamine exposures [153, 155–157]. These diseases are linked by the presence of steatohepatitis. Mechanistically, inflammation in the setting of hepatic steatosis increases ER stress, oxidative damage, mitochondrial dysfunction, and lipid peroxidation, which together drive hepatic insulin resistance [136]. Insulin resistance itself dysregulates lipid metabolism and promotes lipolysis [158], which increases production of toxic lipids, including ceramides, and further impairs insulin signaling, mitochondrial function, and cell viability [136, 159, 160]. Liver disease worsens because ER stress and mitochondrial dysfunction exacerbate insulin resistance [8], lipolysis, and ceramide accumulation [93–95].

Experimental NAFLD with T2DM and visceral obesity is associated with brain atrophy, neurodegeneration, and cognitive impairment [101, 134, 143, 153, 157]. In humans with NASH, the rates of neuropsychiatric disease, including depression and anxiety [161], and risks for developing cognitive impairment [162] are increased. In fact, cognitive impairment and neuropsychiatric dysfunction correlate more with steatohepatitis and insulin resistance than with obesity or T2DM [163, 164]. Therefore, consideration should be given to the potential roles of hepatic insulin resistance and steatohepatitis as mediators of neurodegeneration. To this end, we hypothesized a novel mechanism by which increased levels of cytotoxic ceramides generated in liver could cause neurodegeneration [101, 143, 153, 157]. We also consider visceral fat as another major source of cytotoxic ceramides (Fig. 1.1).

In humans and experimental models of steatohepatitis, irrespective of cause, ceramide gene expression and ceramide levels are increased [16, 17, 134, 165–168]. Furthermore, cultured CNS neuronal cells exposed to short-chain cytotoxic ceramides
Fig. 1.1 High caloric intake and/or chronic low-level nitrosamine exposures (through diet, smoking, and agriculture) promote fatty liver disease (steatohepatitis) that progresses due to injury and inflammation, eventually leading to hepatic insulin resistance. The same poor physiological states also promote obesity, diabetes mellitus, and other peripheral insulin resistance diseases. Toxic lipids, particularly ceramides, made in liver or visceral fat, get released into the circulation, cross the blood–brain barrier, and cause brain insulin resistance, inflammation, energy failure, toxicity, and local production of toxic ceramides. The end result is progressive neurodegeneration, including Alzheimer disease. For a color version of this figure, please refer to the color plate.

developed several of the molecular and biochemical abnormalities that are associated with AD [169,170]. In addition, in vivo administration of the same short-chain toxic ceramides caused cognitive-motor deficits, brain insulin resistance, oxidative stress, metabolic dysfunction, and neurodegeneration, with features similar to those present in AD [168]. Furthermore, recent studies utilizing a brain slice
culture model demonstrated that exposure to long-chain ceramide-containing plasma lipids from obese rats with steatohepatitis, or purified synthetic long-chain ceramides, produced neurotoxic responses with impairments in viability and mitochondrial function [165]. Therefore, toxic lipids generated in the liver can promote or exacerbate neurodegeneration.

**Metabolic Syndrome** Metabolic syndrome is a cluster of disease processes that pivots around insulin resistance, visceral obesity, hypertension, and dyslipidemia [171]. Metabolic syndrome significantly increases risk for coronary artery disease, atherosclerosis, and T2DM, and is frequently associated with NAFLD/NASH, pro-inflammatory and pro-thrombotic states, and sleep apnea [171]. Prevalence rates of metabolic syndrome have increased in recent years in adult populations as well as pediatric populations. Studies have linked peripheral insulin resistance [172], visceral obesity [173], and metabolic syndrome [174–176] to brain atrophy and cognitive impairment with declines in executive function [177]. Although chronic consumption of high caloric, high fat content foods promotes metabolic dysfunction [178], the link to brain aging is still under investigation.

The aggregate findings in human and experimental studies suggest that T2DM, obesity, metabolic syndrome, NAFLD, and probably other peripheral/systemic insulin resistance disease states serve as cofactors in the pathogenesis and progression of neurodegeneration. This suggests that therapeutic strategies to treat systemic insulin resistance may also help reduce progression and severity of AD, but may not prevent it altogether. Correspondingly, studies have already shown that treatment with anti-hyperglycemic or insulin sensitizer agents may reduce AD pathology [179].

### 1.6.4 Nitrosamines and Insulin Resistance

The prevalence rates of Alzheimer disease (AD), obesity, T2DM, NAFLD/NASH, and metabolic syndrome have increased exponentially over the past several decades, and show few hints of plateau [180–184]. The relatively short time interval associated with dramatic shifts in age-adjusted AD morbidity and mortality is consistent with an exposure-related rather than genetic etiology. We have noted that the striking increases in AD mortality rates corrected for age, have given chase to the sharply increased consumption of processed foods, use of preservatives, and demand for nitrogen-containing fertilizers [39]. A common theme resonating from these unnecessary lifestyle trends is that we have inadvertently increased our chronic exposures to nitrosamines (R1N(-R2)-N=O) and related compounds.

**Nitrosamines as Mediators of Disease** Nitrosamines form by chemical reactions between nitrites and secondary amines or proteins. Nitrosamines exert their toxic and mutagenic effects by alkylating N-7 of guanine, leading to increased DNA damage [185] and generation of reactive oxygen species such as superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), which result in increased lipid peroxidation, protein adduct formation, and pro-inflammatory cytokine activation [186]. However, these very same molecular and biochemical pathogenic cascades are associated with major human insulin resistance diseases, including T2D, NASH, and AD [148, 187–192]. The concept that chronic injury caused by exposure to alkylating agents could result in malignancy and/or tissue degeneration is not far-fetched given the facts that: (1) chronic exposure to tobacco nitrosamines causes both lung cancer and emphysema with chronic obstructive pulmonary disease; and (2) exposures to streptozocin (STZ), a nitrosamine-related compound, can cause hepatocellular or pancreatic carcinoma, T2DM, AD-type neurodegeneration, or hepatic steatosis, depending on dose and route of administration [55, 57, 98, 101, 193–197]. Therefore, although research on nitrosamine-related compounds has been largely focused on their mutagenic properties, thorough characterization of their non-neoplastic and degenerative effects is clearly warranted. In this regard, guidance may be obtained from what is already known about STZ-induced disease.

**Epidemiological Evidence that AD Morbidity and Mortality Trends Mimic Exposure-Mediated Diseases** Prior to 1980, epidemiologic trends for AD (increasing prevalence) were opposite those for diabetes mellitus (declining as a cause of death), but over the past three decades, morbidity and mortality
from obesity, T2DM, NAFLD, metabolic syndrome, and AD have trended upward [39], causing them to overlap. Moreover, the rates in which insulin resistance disease prevalence increased correspond with exposure-mediated rather than genetic etiologies. In addition, because the same trends were evident across all age groups, 50 years and older [39], the phenomenon could not be attributed to aging.

**Experimental Link to Nitrosamines** STZ, like other N-nitroso compounds, causes cellular injury and disease by functioning as: (1) an alkylating agent and potent mutagen [57]; (2) an inducer of DNA adducts, including N\(^7\)-methylguanine, which leads to increased apoptosis [198]; (3) a mediator of unscheduled DNA synthesis, triggering cell death [57]; (4) an inducer of single-strand DNA breaks and stimulus for nitric oxide (NO) formation following breakdown of its nitrosamine group [194]; and (5) an enhancer of the xanthine oxidase system leading to increased production of superoxide anion, H\(_2\)O\(_2\), and OH\(^-\) radicals [199]. Ultimately, STZ-induced cellular injury, DNA damage, and oxidative stress cause mitochondrial dysfunction [194], ATP deficiency [200], poly-ADP ribosylation, and finally apoptotic cell death.

The findings of brain insulin deficiency and insulin resistance, deficits in cholinergic function, impairments in spatial learning and memory, and histopathologic lesions corresponding to AD in rats that were given intracerebral injections of streptozotocin [55, 96, 99, 201–204], raised questions about toxin exposures as mediators of AD in humans. This point is strengthened by additional data showing that streptozotocin also causes T2DM and NAFLD [57, 205], and that the degenerative effects of this pro-diabetes drug are mediated by impairments in insulin signaling and energy metabolism, and increased oxidative stress, mitochondrial dysfunction, and cell death.

**Low Levels of Dietary Nitrosamines Cause AD-Type Neurodegeneration** The structural similarities between STZ and nitrosamines, including N-nitrosodiethylamine (NDEA) and N-nitrosodimethylamine (NDMA) [206], together with experimental evidence that high doses of STZ cause cancer whereas lower doses cause diabetes or AD-type neurodegeneration with cognitive impairment [55, 194, 197], led us to hypothesize that although high doses of environmental and consumed nitrosamines cause cancer, exposures to lower, sub-mutagenic doses promote insulin-resistance mediated degenerative diseases, including T2DM, NASH, and AD. Although humans seldom have contact with streptozotocin, they do endure frequent and abundant exposures to structurally related NDEA and NDMA, which are commonplace in highly processed, preservative-laden diets, and therefore problematic. In addition, human exposures to tobacco nitrosamines are also widespread and increased steadily until public health and policy measures blunted tobacco use. It is noteworthy that meta-analysis studies have uncovered links between cigarette smoking and AD [207]. Over the past few decades, Western societies have been assaulted by growing exposures to environmental and food-related nitrosamines. In the United States, the use of nitrate-containing fertilizers and sales of foods preserved with sodium nitrate increased sharply, years prior to the nearly parallel increases in mortality from diabetes mellitus, AD, and even Parkinson disease [39].

These observations led us to test the hypothesis that sub-mutagenic doses of nitrosamine compounds found in processed and preserved foods, e.g. NDEA, cause insulin resistance diseases including neurodegeneration. Alarming, those studies showed that very limited, low-dose exposures to NDEA were sufficient to cause T2DM, NASH, visceral obesity, cognitive impairment, and AD-type neurodegeneration with peripheral, hepatic, and brain insulin resistance [153, 157]. Moreover, the adverse effects of NDEA on these disease processes were exacerbated by chronic high-fat diet feeding which caused visceral obesity and steatohepatitis [96, 166]. Therefore, although high-dose nitrosamine exposures cause cancer, low, limited-dose exposures to the same compounds cause insulin resistance in multiple target organs, including brain. These studies are provocative and support our hypothesis that the relatively recent epidemics of sporadic AD, T2DM, and NASH/metabolic syndrome are mediated by environmental or dietary exposures [39]. This phenomenon could account for the overlapping increases in prevalence of various insulin resistance diseases within the
The apolipoprotein E (ApoE) gene is located on chromosome 19, and is expressed widely throughout the body, but particularly in liver and brain, and its normal functions include lipid transport and metabolism. Three isoforms of ApoE exist: ApoE-ε2, ApoE-ε3, and ApoE-ε4. In FAD2, an ApoE-ε3/ε4 genotype increases risk for AD by 2- to 4-fold, and ApoE-ε4 increases risk for AD by 30-fold relative to ApoE-ε3/ε3 [222]. The presence of one or two ApoE-ε4 alleles is a risk rather than causal mechanism of AD.

FAD2 is a late-onset form of AD (older than 65 years) associated with the ApoE-ε4 genotype (homozygous or heterozygous alleles). FAD3 accounts for 90% of familial early onset AD, and is caused by deletion mutations in the Presenilin 1 (PS1) gene. FAD4 accounts for 5% of FAD [208–210]. Despite their roles as predictors and mediators of AD, it remains unclear how these gene abnormalities or variants mediate their effects, and why aging (at least to middle age) is required for disease to be manifested. Furthermore, concerns have been raised that mechanisms other than AβPP-AB toxicity and deposition mediate neurodegeneration [77]. Herein, we focus the discussion on the potential contributions of these heritable factors to brain aging and insulin resistance.

**FAD1** In FAD1, mutation of the AβPP gene results in aberrant processing of AβPP protein and accumulation of AβPP-AB. The AβPP gene is over-expressed in Down syndrome due to trisomy 21 or translocation of the Down locus [211]. Over expression of the AβPP gene is sufficient to cause early and substantial brain accumulations of AβPP-AB in animal models [212], but other factors, including tau pathology and oxidative stress are required to produce significant cognitive impairment and neurodegeneration [213], and recapitulate the full spectrum of AD as it occurs in humans [214,215].

The Down locus contains a number of genes that are also over-expressed in Down syndrome, and needed to produce the phenotypic features, which include premature aging [216,217]. Familial mutations in the AβPP gene are quite varied and mainly cause cerebral amyloid angiopathy, which is mainly associated with brain hemorrhages rather than dementia [218–220]. However, the FAD1 mutation is linked to early onset AD [78]. Mechanistically, AβPP gene mutations result in aberrant cleavage and accumulation of AβPP-AB, with progressive increases in oxidative stress, neurotoxicity, and activation of kinases that hyper-phosphorylate tau [221]. The chronically increased stress promotes neuroinflammation. These secondary responses may drive AD development by exacerbating normal aging-associated increases in brain insulin resistance.

**FAD2** The apolipoprotein E (ApoE) gene is located on Ch 19. ApoE is expressed widely throughout the body, but particularly in liver and brain, and its normal functions include lipid transport and metabolism. Three isoforms of ApoE exist: ApoE-ε2, ApoE-ε3, and ApoE-ε4. In FAD2, an ApoE-ε3/ε4 genotype increases risk for AD by 2- to 4-fold, and ApoE-ε4 increases risk for AD by 30-fold relative to ApoE-ε3/ε3 [222]. The presence of one or two ApoE-ε4 alleles is a risk rather than causal mechanism of AD.

ApoE is a component of senile plaques and binds to AβPP-AB. ApoE-ε4/ε4 may interfere with AβPP-AB clearance [223, 224]. One study demonstrated a compounded risk for developing AD in hyperinsulinemic diabetics who carried at least one ApoE-ε4 allele, and relative resistance to AD among ApoE-ε4 negative non-diabetics [225]. Correspondingly, ApoE-ε4 positive, hyperinsulinemic diabetics have higher AβPP-AB plaque burdens compared with ApoE-ε4 negative individuals [226]. These findings suggest co-morbid interactive roles of T2DM/insulin resistance and ApoE-ε4 in the pathogenesis of AD, although results of the Rotterdam study failed to support this concept [227]. Furthermore, the correlation between ApoE-ε4 genotype and elevated HOMA-IR (homeostasis model of assessment-insulin resistance) [228] suggests that the ApoE-ε4 allele also increases risk for developing peripheral insulin resistance or T2DM. This phenomenon could explain the increased risks for developing AD (brain diabetes) with aging and an ApoE-ε4 genotype.

**FAD3 and FAD4** FAD3 in caused by autosomal dominant deletion mutations in the Presenilin 1 (PS1) gene located on gene on Ch14. FAD3 accounts for...
the vast majority of early onset familial AD, and its penetrance approaches 100%. FAD4 is caused by sequence variations or polymorphisms in the PS2 gene, which is located on Ch1q31-q42.

Presenilins encode gamma-secretases, which form a complex with nicastrin, Aph1, and PEN-2. Presenilins cleave both AβPP and Notch 1, and have functional roles in synaptic plasticity [229]. In AD, AβPP is cleaved by γ-secretases, followed by γ-secretase to generate AβPP-AB fibrils that accumulate as toxic oligomers or aggregate into plaques. AβPP-AB toxic fibrils cause inflammation, synaptic loss, abnormal phosphorylation of tau, and cell death. However, Presenilins also downregulate expression of the insulin receptor and signaling through gamma secretase-independent pathways [230]. Mechanistically, GSK-3β phosphorylation of PS1 inhibits insulin receptor transcription and insulin receptor gene expression [231]. Therefore, PS1-mediated impairments of brain insulin signaling could represent a major causal factor in FAD3 and possibly FAD4-associated neurodegeneration and dementia.

1.7 MECHANISTIC HYPOTHESES REGARDING AD PATHOGENESIS

1.7.1 Reverberating Mal-signaling Network

Chronic obesity, T2DM, NASH, and AD share in common insulin resistance that is associated with inflammation and lipid dyshomeostasis. Chronic inflammation is mediated by activation of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) [232–234]. Lipid dyshomeostasis results in increased ceramide generation in adipose tissue and liver [169, 235–237]. Insulin resistance, inflammation, and ceramide accumulation promote oxidative and ER stress, which impair mitochondrial function, energy balance, and membrane integrity, and worsen insulin resistance, inflammation, and ceramide generation [93, 94, 238, 239] [240, 241]. Unchecked, the rates of injury eventually exceed those of repair. Resulting chronic insulin resistance disease states initiate a harmful positive feedback mal-signaling loop, whereby interconnected pathophysiologic processes cause progressive organ-system degeneration.

1.7.2 Extrinsic Factors Mediating Brain Insulin Resistance—the Liver–Brain Axis of Neurodegeneration

Human and experimental animal studies demonstrated that obesity, T2DM, NASH, and metabolic syndrome were associated with cognitive impairment, brain insulin/IGF resistance, and neurodegeneration. Further investigations showed that the peripheral insulin resistance states that lead to brain atrophy and brain insulin resistance share in common steatohepatitis and/or visceral obesity with increased ceramide accumulation [159, 234, 238, 242]. Similarly, steatohepatitis caused by limited, low-level nitrosamine exposures plus high-fat diets lead to hepatic insulin resistance associated with ceramide accumulation [55, 101, 153, 157]. To understand the significance of these connections to ceramides, a brief review of ceramide biochemistry and physiology is needed.

Ceramides: Lipid Signaling and Lipotoxicity

Ceramides are lipid-signaling molecules [236, 243] that regulate diverse positive (growth, motility, adhesion, differentiation) and negative (senescence, apoptosis, insulin resistance) cellular functions. Ceramides participate in membrane lipid microdomains, i.e. rafts [244], enabling them to regulate responses to stress [245–247]. Ceramides accumulate in cells due to disturbances in sphingolipid metabolism [234, 248–250] and upregulation of pro-ceramide genes [134, 251]. Alterations in sphingolipid metabolism lead to aberrantly increased intracellular levels of ceramides mediate insulin resistance [136, 159, 248, 249, 252–254] in obesity, T2DM, NASH, and AD [93, 94, 169, 235–241].

Ceramides and Insulin Resistance

Ceramides achieve their inhibitory effects on insulin signaling and cellular physiology by inhibiting PI3 kinase-Akt [255, 256], and activating pro-inflammatory cytokines [234, 257, 258], pro-apoptotic mechanisms [249], GSK-3β [259, 260], protein phosphatase 2A [254], and phosphatase and tensin homologue deleted on chromosome 10 (PTEN) [261]. In addition, increased ceramide load is associated with ER and oxidative stress, which worsen insulin resistance [262–264].
Ceramides and the Liver–Brain Axis of Neurodegeneration  Because none of the experimental models of peripheral insulin resistance were associated with substantial alterations in brain ceramide gene expression or enzymatic activity, to invoke a role for ceramides as mediators of brain atrophy, cognitive impairment, and insulin resistance, alternative mechanisms must be considered. We hypothesize that in settings of obesity, T2DM, NASH, and metabolic syndrome, cognitive impairment and neurodegeneration are mediated by factors pertaining to the common occurrences of steatohepatitis and peripheral ceramide accumulation.

The proposed mechanism predicts the existence of a “liver–brain axis of neurodegeneration” whereby cytotoxic ceramides generated in liver and probably also visceral fat, leak into peripheral blood following local tissue inflammation, injury, and cell death. Cytotoxic ceramides then traffic through the circulation, and due to their lipid soluble nature, cross the blood-brain barrier and exert neurotoxic and neurodegenerative effects by impairing insulin signaling [16, 167, 265] and activating pro-inflammatory cytokines [234, 257]. This proposed scheme explains how brain insulin resistance, which is an early and important feature of AD, could be mediated by peripheral insulin resistance diseases that are associated with hepatic or visceral fat accumulation, inflammation, dysregulated lipid metabolism, ER/oxidative stress, mitochondrial dysfunction, and activation of pro-death signaling networks [165, 167, 265].

Human and Experimental Studies Supporting the Liver–Brain Axis Model of Neurodegeneration  In support of this concept, mass spectrometry-based lipidomic studies of plasma revealed elevated levels of saturated sphingolipids (N16:0 and N21:0) in AD relative to control subjects, and also linked severity of cognitive impairment to altered levels of specific very long chain ceramides [266]. Other studies showed that elevated levels of very long-chain saturated ceramides (C22:0 and C24:0) in plasma predict memory loss and hippocampal atrophy in MCI [267], whereas increased ratios of dihydrosphingomyelin to dihydroceramide and sphingomyelin to ceramide correlate with slower progression of AD [268]. A third line of evidence stems from the finding that insulin sensitizer mediated reductions in oxidative stress [101, 269] and ceramide levels were associated with preservation of cognitive function and brain volume in an experimental model of diet-induced obesity with T2DM and steatohepatitis [270].

Additional in vitro and in vivo experiments demonstrated that: (1) exposure of human CNS neuronal cells or rat brain slice cultures to cytotoxic ceramides caused mitochondrial dysfunction, insulin resistance, and cell death; (2) cytotoxic ceramides injected into peripheral blood were recovered in brain tissue and demonstrated to cause neurocognitive impairment, brain insulin/IGF resistance, molecular abnormalities in gene and protein expression as occur in AD, and increased oxidative stress; (3) lipids isolated from liver or peripheral blood of obese/diabetic experimental animals with steatohepatitis and insulin resistance were neurotoxic to slice cultures; and (4) the spectrum of long chain ceramides found in liver and peripheral blood from humans and experimental animal models with insulin resistance caused mitochondrial dysfunction and neuronal death in cultured CNS cells. Altogether, the findings suggest that in insulin resistance disease states, cytotoxic ceramides from hepatocytes, and probably also visceral adipocytes, leak into peripheral blood and exert toxic and degenerative effects in the brain by impairing insulin signaling. We suggest that by preventing this process, rates of neurodegeneration in setting of peripheral insulin resistance would be reduced. Moreover, by detecting elevated levels and altered ceramide profiles in peripheral blood, we may be able to identify individuals at risk for later development of cognitive impairment.

1.7.3 Intrinsic Pathway to Type 3 Diabetes  Although T2DM, obesity, NASH, and metabolic syndrome are major driving forces in the cognitive impairment and AD epidemics, it is important to bear in mind that most cases of AD are not associated with obesity or significant peripheral insulin resistance diseases. Yet, AD is clearly associated with brain insulin/IGF resistance and deficiency, and experimentally, neurodegeneration with brain insulin resistance can be produced in the absence of peripheral insulin resistance. However, like T2DM, NASH,
and metabolic syndrome, brain-restricted insulin/IGF resistance in AD and experimental models is associated with dysregulated lipid metabolism, long-chain ceramide accumulation, inflammation, ER and oxidative stress, and mitochondrial dysfunction [48, 165].

Previously in this review, the role of aging was discussed and determined to be critical but insufficient to serve as the sole cause of neurodegeneration. With regard to FAD, evidence has been presented that presenilin and AβPP gene mutations and the ApoE-ε4 allele exert their effects, in part, by impairing insulin signaling in the aging brain. Although the causes of primary brain insulin/IGF resistance and deficiency in sporadic AD are not known, experimental evidence suggests roles for nitrosamine exposures. This concept fits with data indicating widespread and abundant exposures to nitrosamines and their precursors in our diets and resulting from lifestyle trends throughout the past 50 years. Our experiments have shown that low-level nitrosamine exposures can cause the full spectrum of insulin resistance diseases that plague our society, including T2DM, visceral obesity, NASH, metabolic syndrome, and AD-type neurodegeneration. These findings led us to the concept of an intrinsic pathway for neurodegeneration. In essence, we propose that AD and probably other neurodegenerative diseases are mediated by chronic, low-level exposures to nitrosamines, through diet and lifestyle choices, and that these toxins exert their degenerative effects by causing insulin resistance and oxidative stress in various organs, including the brain. In addition, nitrosamine exposures exacerbate the effects of obesity and aging-associated insulin resistance, and thereby serve to initiate, propagate, and exacerbate the AD neurodegeneration cascade.

1.8 CONCLUSION

Brain insulin/IGF resistance, whether primary or secondary, initiates a cascade driven by increased oxidative stress, neuro-inflammation, impaired cell survival, mitochondrial dysfunction, dysregulated lipid metabolism, and ER stress. These processes compromise neuronal and glial functions, reducing neurotransmitter homeostasis, disrupting neuronal cytoskeletal and AβPP functions, and causing toxic oligomeric fibrils and insoluble aggregates (neurofibrillary tangles and AβPP-Aβ plaques) to accumulate.

AD progresses due to: (1) activation of a harmful, self-reinforcing, positive feedback loop that progressively worsens the effects of insulin resistance; and (2) the formation of ROS- and RNS-related lipid, protein, and DNA adducts that permanently damage basic cellular and molecular functions. Given the fact that many of the fundamental molecular and cellular abnormalities in AD also occur in Parkinson disease, motor neuron disease, alcoholic brain disease, and others, perturbations in sphingolipid metabolism with accumulation of neurotoxic ceramides may be a common factor in the pathogenesis and progression of various neurodegenerative diseases [48, 165, 167, 170, 271].

Because the underlying cellular, molecular, and biochemical abnormalities identified in various insulin/IGF resistance diseases are nearly identical, we hypothesize that they are actually manifestations of a single disease in which different organs and tissues can be targeted. A similar phenomenon occurs with respect to atherosclerosis, which worsens with aging and causes either distinct or overlapping patterns of organ dysfunction, depending upon the affected arteries. By regarding the seemingly complex and multifaceted array of chronic insulin resistance diseases as a single pathologic process with multiple targets, we broaden opportunities to discover and implement new treatments and preventive measures to conquer these epidemics. In this regard, we presented evidence that chronic environmental/dietary exposures to nitrosamines and possibly other toxins, together with poor lifestyle choices are responsible for the collective epidemic insulin resistance diseases. In our opinion, these diseases are preventable.

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