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# Alzheimer's Disease and Diabetes

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## 1. Introduction

*"I have lost myself"*

- *Auguste Deter, the first patient diagnosed with Alzheimer's Disease, 1906*

**Identification of Alzheimer's Disease** Alois Alzheimer was a German neuropathologist and among the first to identify and describe the hallmarks of what is known today as Alzheimer's disease (AD). In November of 1901, Dr. Alzheimer was presented with 51 year-old Auguste Deter who was suffering from mental incompetence, aphasia, disorientation, paranoia, and unprovoked bursts of anger. Deter's emotional and mental devastation became evident when she confided to Dr. Alzheimer "I have lost myself."

Symptoms similar to Deter's had been observed in patients for years and were considered a natural part of aging. However, it was unusual for such a pointed disease state to occur in someone so young. Over the next four and half years, Deter became increasingly demented, until her death at the age of 55. Upon examination of Deter's brain, Dr. Alzheimer found microscopic strands of protein which he described as "tangled bundles of fibrils" (neurofibrillary tangles) in addition to "miliary foci" (amyloid plaques). In 1906, at the 37<sup>th</sup> Conference of South-West German Psychiatrists in Tübingen, Alois Alzheimer presented Deter's case as, "a peculiar disease of the cerebral cortex."

To this day both the cause of and treatment for AD remain a mystery. AD is a multifaceted disease of great complexity, however, over 100 years of research has provided clues to its mechanisms. Of particular recent interest is the emerging realization that another rapidly growing disease, type 2 diabetes mellitus (T2DM), is linked to development of AD [1].

This chapter examines the current state of knowledge regarding the association of T2DM to vascular changes in the brain and the implications these changes have in AD development.

Other factors that contribute to AD such as insulin resistance and accumulation of the neurotoxic peptide amyloid beta ( $A\beta$ ) are also examined. It's likely that no central cause of AD exists but rather, the disease represents a breakdown of several critical components involved in the general health and function of the brain.

**Epidemiology of AD and T2DM** AD is the most common form of dementia [2] and remains incurable. While the cause of AD remains unknown, several risk factors have been identified that may provide insight into the fundamentals of AD pathogenesis.

T2DM is a known risk factor for AD [1] suggesting that insulin signaling abnormalities play a central role in AD pathology. Moreover, AD brains show decreased insulin levels, decreased activity of insulin receptors and signs of compensatory mechanisms such as increased insulin receptor density [3] indicating AD as "type 3 diabetes" [4, 5].

Loss of insulin signaling in diabetes can occur by either type 1 or type 2 processes. Type 1 diabetes mellitus (T1DM) is characterized as an autoimmune disease that results in the destruction of insulin producing  $\beta$  cells found in the pancreas. In contrast, T2DM is a state of insulin resistance in which insulin levels are normal or elevated but tissues are unresponsive to its effects. While both T1DM and T2DM can lead to cognitive deficits, T2DM poses a greater risk for AD development [6, 7] and as a result the parallels between T2DM and AD are studied more vigorously than T1DM associations. Therefore, the majority of information presented here pertains to type 2 diabetic pathologies.

In addition to insulin resistance, T2DM is associated with the development of vascular dysfunction in the brain [8, 9]. T2DM is a risk factor for microvascular complications as well as macrovascular defects [10] such as stroke [11]. Vascular abnormalities are strongly associated with AD [12-16] implying further involvement of T2DM in disease onset.

## 2. Type 2 diabetes, vascular changes and Alzheimer's disease

**Insulin signaling in the vasculature** Activation of the insulin receptor (IR) leads to phosphorylation of insulin receptor substrate (IRS) which serve as docking proteins for phosphatidylinositol 3-kinase (PI3K). PI3K generates phosphatidyl-3,4,5-triphosphate ( $PIP_3$ ) which then phosphorylates 3-phosphoinositide-dependent protein kinase-1 (PDK-1). Finally, PDK-1 phosphorylates Akt and stimulates endothelial nitric oxide synthase (eNOS) resulting in the production of nitric oxide (NO) and vascular relaxation [17, 18]. Interestingly, insulin receptor activation can also mediate vasoconstriction. Activation of IR can also lead to phosphorylation of Shc which then binds Grb-2 resulting in activation of Sos. This complex then activates Ras leading to phosphorylation Raf which results in activation of MAPK. Activation of MAPK stimulates release of endothelin-1 (ET-1), a vasoconstrictor [19-21]. By mediating vascular properties, insulin signaling plays a significant role in glucose and oxygen availability to the brain. Conversely, dysfunction in insulin signaling, as observed in T2DM, has profound detrimental effects on hemodynamics and, thus, maintenance of normative brain function.

**Vascular complications associated with type 2 diabetes** It is estimated that approximately 200 million people worldwide have diabetes and by 2025 the number is expected to increase to 333 million [22]. Epidemiological studies have indicated that patients with T2DM have a greater incidence of cardiovascular disease, cerebrovascular disease (CVD), hypertension and renal disease relative to the general population [8, 9]. In addition, a large number of population-based studies have identified diabetes as a risk factor for dementia [23-25], primarily as a result of CVD [26, 27]. At only 3% of body weight, the brain uses ~20% of the body's oxygen and ~25% of the body's blood glucose [28, 29], demonstrating that it is by far the most metabolically active organ. This oxygen and glucose consumption is constantly required, since brain neurons are obligate aerobic cells and have no other source of energy. The majority of this energy is used to maintain cellular ionic homeostasis, and thus when cerebral blood flow (CBF) ceases, brain function ends within seconds and damage to neurons occurs within minutes [30].

The vascular complications associated with diabetes can be divided into two classes based on the vascular etiology of their pathology: macrovascular (hypertension, coronary artery disease, atherosclerosis, stroke) and microvascular (neuropathy, retinopathy, nephropathy). Macrovascular complications are those that affect the larger (non-capillary) blood vessels. Statistics show that diabetes increases the risk of stroke and atherosclerosis [31]. Atherosclerosis accounts for 70% of morbidity associated with T2DM [32], while other studies have shown an association between the degree of hyperglycemia and increased risk of myocardial infarction and stroke [33-36]. While macrovascular complications themselves represent important pathological consequences of T2DM, they have also been shown to provide the etiological link between T2DM and the development of Alzheimer's disease.

**Link between type 2 diabetes and Alzheimer's disease** AD is an age-related disorder characterized by progressive cognitive decline and dementia. An estimated 5.3 million people in the United States are currently affected and represents the sixth-leading cause of death. Significant evidence has been provided that links T2DM to AD. For example, a comprehensive meta-analysis showed that the aggregate relative risk of AD for people with diabetes was 1.5 (95%-CI 1.2 to 1.8) [37]. Studies have shown that T2DM, impaired fasting glucose and increased islet amyloid deposition are more common in patients with Alzheimer's disease than in control subjects [38, 39]. Unsurprisingly, insulin signaling provides an important mechanistic link between T2DM and AD.

Ischemic CVD caused by T2DM is positively associated with AD through shared pathological mechanisms such as hyperinsulinemia, impaired insulin signaling, oxidative stress, inflammatory mechanisms and advanced glycation end-products (AGEs) [40]. Defective insulin signaling is associated with decreased cognitive ability and development of dementia, including AD [41], rendering signaling neurons more vulnerable to metabolic stress and accelerating neuronal dysfunction [42]. In vitro insulin-stimulated Akt phosphorylation is decreased in hyperinsulinemic conditions in cortical neurons [43]. Finally, all forms of amyloid beta ( $A\beta$ ) (monomers, oligomers and  $A\beta$ -derived diffusible ligands (ADDLs)) can inhibit insulin signaling by directly binding to the insulin receptor and inhibit insulin signaling [44].

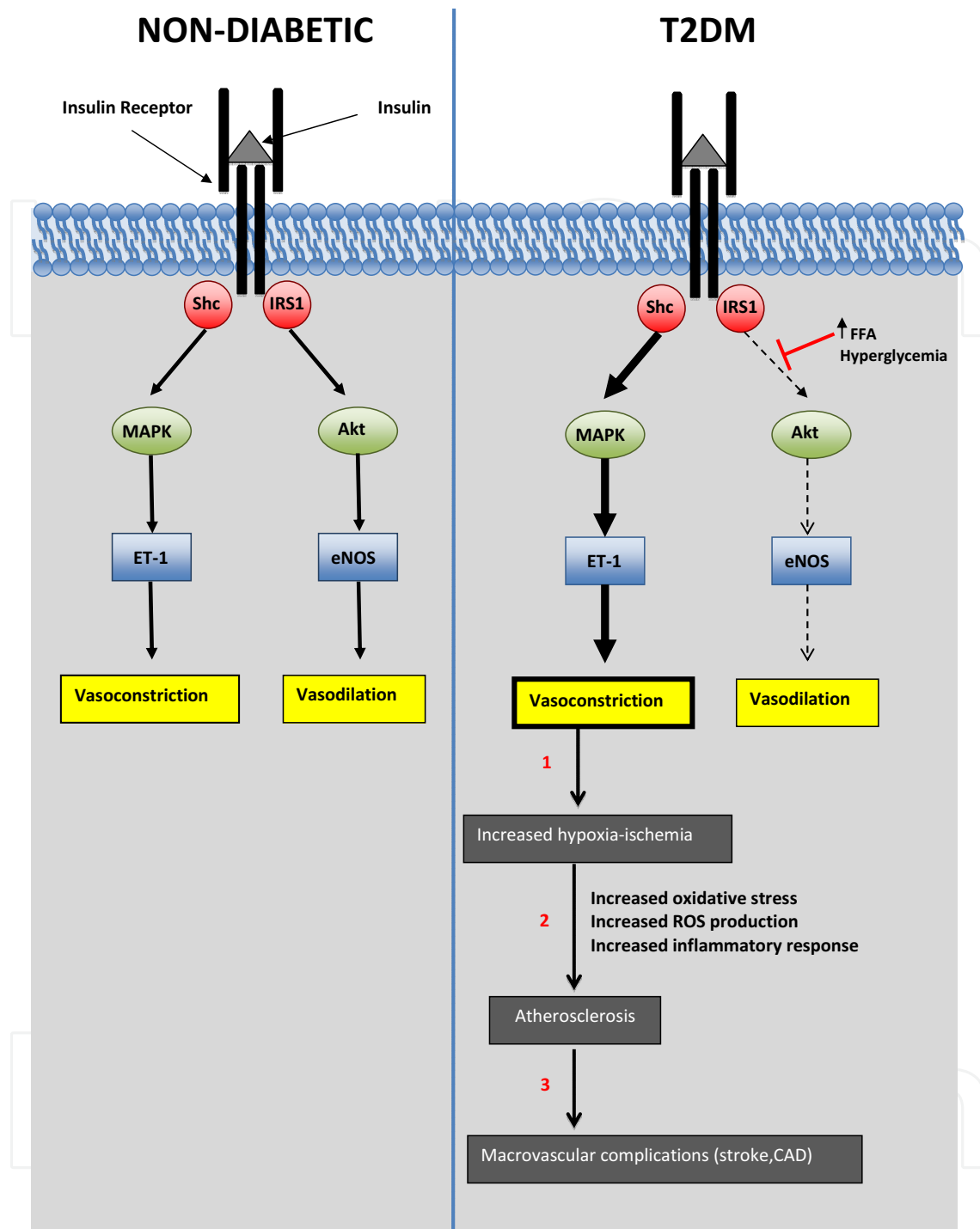
**Mechanisms of macrovascular complications of diabetes** A central pathological mechanism in diabetic-related macrovascular disease is atherosclerosis, which leads to the hardening of

arterial walls throughout the body resulting in impaired blood flow. Although the mechanism for the susceptibility of diabetic patients to ischemic heart disease remains unclear, accumulating lines of evidence implicate hyperglycemia, hyperlipidemia and inflammation as playing key roles in the development of this disorder [45]. This link between obesity and both T2DM and atherosclerosis implicates elevated amounts of glucose oxidized LDL and free fatty acids (FFAs) in disease pathogenesis, potentially as triggers for the production of pro-inflammatory cytokines by macrophages [32].

In the insulin resistant state, there is a specific impairment in the vasodilatory PI3K pathway, whereas the Ras/MAPK-dependent pathway is unaffected [46, 47]. This results in decreased production of NO and an increased secretion of ET-1 in humans [48] leading to increased vasoconstriction. The decrease in NO production is significant in that NO protects blood vessels from endogenous injury by mediating molecular signals that prevent platelet and leukocyte interaction with the vascular wall and inhibit vascular smooth muscle cell proliferation and migration [49, 50]. Decreased production of NO allows for increased expression of proinflammatory transcription factor NF- $\kappa$ B, and subsequent expression of leukocyte adhesion molecules and production of chemokines and cytokines [51]. Activation of these proteins promote monocyte and vascular smooth muscle cell migration into the intima and formation of macrophage foam cells, initiating the morphological changes associated with the onset of atherosclerosis [52, 53].

High levels of FFAs are found in insulin-resistant individuals. FFAs generated by increased activity of hormone-sensitive lipase that contribute to and result in insulin resistance [54-56]. In vitro vascular endothelial cell culture treated with FFA resulted in decreased insulin-stimulated eNOS activity and NO production [57]. It is believed that FFA increases cellular levels of diacylglycerols, ceramide, and long-chain fatty acyl coenzyme A (CoA), all of which have been shown to activate protein kinase C (PKC $\beta$ 1). Activation of PKC $\beta$ 1 results in increased phosphorylation of IRS-1 that leads to reduced Akt and eNOS resulting in decreased vasodilatory capacity [58, 59]. Increase in FFAs result in an increase in reactive oxygen species (ROS) from NADPH and the mitochondrial electron transport chain [60]. The increase in ROS results in increased PKC which activates the hexosamine biosynthetic pathway leading to increased AGEs and subsequent decrease in endothelial-derived NO [60]. Hyperglycemia has been found to decrease activation of Akt and eNOS via O-GlcNAC of eNOS at the Akt phosphorylation sites [61, 62]. Hyperglycemia increases activation of PKC $\alpha$ , PKC $\beta$ , PKC $\delta$  resulting in decreased eNOS and concomitant increase in endothelial ET-1 [60]. T2DM is associated with vascular dysfunction as a result of increased atherosclerosis and decreased cerebral blood flow. The combination of both processes is decreased glucose and oxygen supply to vital organs such as the brain. The biochemical events leading to the macrovascular impairment has particular significance to brain health as the risk of stroke is a major complication of T2DM.

**Type 2 diabetes and cardiovascular disease** T2DM has been shown to be associated with an increased risk of coronary heart disease and stroke [63-66]. Insulin resistance, the mechanism underlying T2DM, has also been linked to a higher incidence and recurrence of stroke [67]. Two key pathological mediators of stroke observed in T2DM are intracranial stenosis [68] and



**Figure 1.** Pathways leading to macrovascular complications of type 2 diabetes mellitus (T2DM). In non-diabetic individuals (*left*), activation of the insulin receptor can result in activation of both vasodilatation and vasoconstriction. Under normative conditions, there is a balance of both processes to regulate the immediate metabolic requirements of various tissues. In type 2 diabetic patients (*right*), factors such as an increase in free fatty acids and hyperglycemia have been shown to specifically inhibit the Akt pathway while the MAPK pathway remains unaffected. This leads to an imbalance in homeostatic regulation of vascular function and hemodynamics (1). The resultant decrease in nutrient availability to affected tissues results in an increase in oxidative stress and ROS production and an increased inflammatory response (2). Released pro-inflammatory cytokines and macrophage recruitment instigates the onset of atherosclerosis, ultimately leading to macrovascular complications (3).



carotid atherosclerosis [69]. Insulin resistance has been associated with elevated expression of the fibrinolytic inhibitor plasminogen activator inhibitor 1 [70] resulting in decreased fibrinolytic capacity and concurrent increased thrombosis due, in part, to an increase in platelet activation [71]. Insulin resistance has also been shown to induce endothelial dysfunction and inflammation [71], adversely affecting vascular function and initiating atherosclerosis, respectively. Collectively, these data implicate insulin resistance to the impairment of normative cerebrovascular function resulting in the activation of pathways that encourage the onset of stroke. Stroke could, in turn, exacerbate and/or initiate the onset of another disorder such as AD.

Pre-existing CVD has been identified as a significant risk factor for AD. The vascular hypothesis of AD posits that vascular dysfunction, such as stroke, is a pre-requisite for the development of this disorder. It has been reported that the risk of AD is three times greater after the occurrence of stroke [72]. Stroke may result in neurodegeneration [73, 74], resulting in the rapid cognitive decline observed in AD patients [75]. It has even been proposed that stroke may be the underlying cause of 50% of AD cases [74]. Conversely, individuals presenting with severe cognitive impairments, and possibly AD, may be at a greater risk for the development of stroke or CVD [76, 77].

The amyloid hypothesis of AD was long held as the prevailing theory explaining the etiology of AD. However, emerging evidence compiled from the last 20 years has suggested that the pathology associated with AD is vascular in origin. The vascular hypothesis of AD states that pre-existing cardiovascular dysfunction such as stroke, hypertension and atherosclerosis results in chronic cerebral hypoperfusion that could encourage the onset of AD. Several lines of evidence have been provided in support of this hypothesis. For example, it has been shown that cerebrovascular dysfunction precedes cognitive decline and the onset of neurodegenerative changes in AD and AD animal models [12, 13]. In rhesus monkeys, dystrophic axons labeled with amyloidogenic enzyme, BACE1, were found in close proximity or in direct contact with cortical blood vessels [78], asserting a tight association with AD pathology and vascular dysfunction. Clinical and epidemiological evidence provides further support of the vascular hypothesis.

AD patients show a greater degree of vascular narrowing of carotid arteries [65] and cerebral arteries of the Circle of Willis [79, 80]. In addition, large artery CVD was positively correlated to the frequency of neuritic plaques [81]. Several vascular risk factors such stroke (silent infarcts, transient ischemic attacks), atherosclerosis, hypertension, heart disease (coronary artery disease, atrial fibrillation) and diabetes mellitus have been associated with an increased risk AD-type dementia [82]. Between 60 to 90% of AD patients exhibit various cerebrovascular pathologies including White matter lesions, cerebral amyloid angiopathy (CAA), microinfarcts, small infarcts, hemorrhages and microvascular degeneration [12-16]. It is believed that cardiovascular dysfunctions act as a nidus for accelerated A $\beta$  deposition resulting in the onset of AD [83].

Aberrant blood brain barrier (BBB) function exposes neurons to neurotoxic substances. Chronic cerebral hypoperfusion is believed to render the brain more vulnerable to various insults, resulting in AD and associated cognitive loss [84]. Clinical observations in AD patients

have revealed extensive degeneration of endothelium [85] and features indicative of BBB breakdown [86]. At the cellular level, AD is known to cause abnormal structural changes to arterioles and capillaries, swelling and increased number of pinocytotic vesicles in endothelial cells, decreased mitochondrial content, increased deposition of proteins of the basement membrane, reduced microvascular density and occasional swelling of astrocyte endfeet [87-92]. A $\beta$  trafficking across the BBB deposition is also dependent on mechanisms of influx and efflux. Increased expression of receptor for advanced glycation endproducts (RAGE) may be responsible for A $\beta$  influx from the blood to the brain has been reported in addition to a decrease in LRP1 receptors that are responsible for clearing A $\beta$  from the brain to the blood [12, 93].

A functional consequence associated with BBB dysfunction is the resultant impairment in cerebral hemodynamics. AD impairs autoregulation, the mechanism that is responsible for the stabilization of blood flow to the brain in response to changes in cerebral perfusion pressure [94]. In an APP  $\times$  PS1 mouse model neurovascular coupling, the process in which activation of a brain region evokes a local increase in blood flow, was impaired [95]. Finally, AD has shown to adversely affect vasomotor/vascular reactivity, the process that mediates vasodilatory or vasoconstrictor responses of cerebral blood vessels to hypercapnic or hypocapnic stimuli (ie. global or regional brain blood flow response to systemic changes in arterial CO<sub>2</sub>) [96-98]. Cumulatively, the impairment of these processes adversely affects cerebral blood regulation that, in turn, would negatively affect nutrient availability to neurons. This would result in cerebral hypoperfusion, a process that is widely believed to initiate the onset of AD pathology.

There are a number of known direct links between biochemical pathways central to AD and hypoxia/ischemia. A rat model for vascular cognitive impairment has been developed referred to as the two-vessel occlusion model of cerebral ischemia. Studies found decreased cerebral blood flow up to 4 weeks, cognitive deficits, APP proteolysis to form A $\beta$ -sized fragments [99-101]. Other studies have observed an overexpression of A $\beta$  persisting for up to 3 months after surgery [102] and cognitive impairment [103], strongly suggesting that decreased CBF is a key mediator in the pathophysiology of AD. Several studies have been able to identify some of the molecular mechanisms as to how hypoxia/ischemia exerts its effects on AD-related genes.

APP expression increases following chronic cerebral hypoperfusion and ischemia [104, 105], and a greater proportion of APP is proteolytically cleaved by increased activity of amyloidogenic enzyme, BACE1, which is concurrently increased in AD following ischemic events [106]. Hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) plays an essential role in cellular and systemic responses to low oxygen and has been found to increase BACE1 mRNA expression [107]. Furthermore, BACE1 stabilization is enhanced in AD in addition to a decrease in its trafficking [108, 109]. Increased BACE results in greater  $\gamma$ -secretase-mediated production of A $\beta$  [110]. In an APP overexpressing mouse model, chronic cerebral hypoperfusion as the result of cerebral amyloid angiopathy (pathological deposition of A $\beta$ <sub>1-40</sub> in brain blood vessels) was followed by an increased rate of leptomeningeal A $\beta$  precipitating the risk of microinfarcts [111]. Hypoxia/



ischemia not only causes increased amyloidogenic cleavage of APP and greater A $\beta$  production, but also impairs A $\beta$  degradation and trafficking [12, 112].

Decreased A $\beta$ -degrading enzymes in response to hypoxic conditions increase the likelihood of developing pathological levels of A $\beta$  in the brain [113-115]. A $\beta$  serves not only as the end result of a pathological cascade, but A $\beta$  itself has been found to contribute to dysfunction in components of the neurovascular unit. In endothelial cells A $\beta$  was observed to decrease endothelial cell proliferation and accelerate senescence of endothelial cells *in vivo* and *in vitro*, inhibit VEGF-induced activation of Akt and eNOS in endothelial cells [116, 117]. A $\beta$  has been found to decrease eNOS (via PKC-dependent pathway) resulting in decreased vascular tonus and decreased substance P-induced vasodilation of the basilar artery [118, 119]. In vascular smooth muscle cells (VSMCs), A $\beta$  affects cellular morphological changes [120] and increases expression of transcription factors, serum response factor and myocardin, resulting in decreased A $\beta$  clearance by downregulating LRP expression [12]. Finally, A $\beta$  has been shown to cause retraction and swelling of astrocyte endfeet in an AD mouse model with CAA [121] as well as increase cholinergic denervation of cortical microvessels which, taken together, results in impaired functional hyperemia [122].

**Type 2 diabetes and vascular dementia** A significant number of population-based studies have indicated an increased risk for the development of dementia attributed to T2DM [23-25]. Due to the importance of insulin in the regulation of several cardiovascular functions, it is unsurprising that insulin resistance plays a role in the cerebrovascular mechanisms of T2DM-induced dementia. The presence of brain infarcts in demented diabetics who did not have AD has been reported [123]. Interestingly, the association between T2DM and the development of AD and VaD has been found to be independent of hypertension and hypercholesterolemia [23] indicating that is CVD alone is not sufficient to initiate dementia. Non-cerebrovascular mechanisms such as peripheral hyperinsulinemia and generation of advanced glycation end-products also play in the etiology of T2DM-related dementia [124]. Studies have shown that the increased risk of developing vascular dementia was greater than developing AD in type 2 diabetics [7, 125, 126], indicating that although symptomatically similar and frequently confused [127], their etiologies are distinct.

**Vascular dementia versus Alzheimer's dementia** The leading cause of dementia is Alzheimer's disease accounting for 70-90% of all cases [127], while vascular dementia (VaD) accounts for the majority of the remaining incidents of dementia [128]. They share common risk factors including hypertension, diabetes mellitus, and hyperlipidemia. [129], highlighting the tight association between these two forms of dementia. In fact, it is now widely believed that AD and VaD are frequently present in the same brain. So-called "mixed dementia" has been observed in elderly people with cardiovascular risk factors in addition to slow progressive cognitive decline [130].

Differing clinical manifestations separate VaD from AD dementia. For example, VaD progression appears more varied than AD in relation to symptoms, its rate of progression and the disease outcome [131]. Increased damage to the ganglia-thalamo-cortical circuits specific to VaD results in problems with attention and the planning and speed of mental processing whereas the primary impairments characteristic of AD are memory and language-related

[132]. It has been suggested that differences in the clinical observations in AD and VaD patients may be due to the type, severity and location of vascular damage [133-135]. Furthermore, perturbations in vascular hemodynamics have been observed in VaD and AD [136, 137], however, AD patients had comparatively less impairment in cerebral perfusion than those with VaD [138] suggesting that hemodynamic disturbances may underlie different types of dementia [138]. While the precise mechanism that vascular risk factors initiate cognitive decline remains elusive [139], T2DM have been identified as an important contributing factor to the development of VaD.

**Associations between vascular dementia and Alzheimer's dementia** While regarded as two separate conditions, AD and VaD share common cerebrovascular pathologies such as CAA, endothelial cell and vascular smooth muscle cell degeneration, macro- and microinfarcts, hemorrhage and white matter changes [140-142]. These shared pathologies have been shown epidemiologically with almost 35% of AD patients showing evidence of cerebral infarction at autopsy [143, 144], and, conversely, VaD patients display AD-like pathology in the absence of pre-existing AD [145]. It has been postulated that CVD, thought to be the etiology of both disorders, not only result in dementia but also increase the likelihood of individuals with AD-related lesions for developing dementia [146, 147].

### 3. Insulin signaling in the brain

**Insulin/IGF-1 pathway activation.** The brain is a major metabolic organ that accounts for ~25% of the body's total glucose use [28, 29]. While glucose uptake in peripheral tissues requires insulin, in the brain this is considered to be an insulin-independent process. Insulin, however, along with Insulin-like Growth Factor-1 (IGF-1), are required for proper brain function as they provide critical neurotrophic support for neurons. IGF-1 and insulin share similar amino acid sequences/ tertiary structures [148] and are known to bind to and activate one another's receptors [149]. Both insulin and IGF-1 receptors are tyrosine kinases [150-152] that, when activated, phosphorylate substrate proteins such as IRS. IRS phosphorylation leads to downstream activation of PI3K and Akt, a serine/threonine kinase and key mediator of insulin/IGF-1's neurotrophic effects. Neuronal processes known to be, at least in part, under the control of insulin/IGF-1 include regulation of apoptotic proteins, transcription of both survival and pro-death genes, neurite outgrowth, and activity of metabolic proteins.

The source of brain insulin remains controversial. While preproinsulin mRNA has been reported in the neurons [153-155], very little insulin is synthesized in the brain [156]. Additionally, glial cells have been found not to be involved in insulin production [157], therefore, it is recognized that the majority of insulin in the brain is produced by pancreatic  $\beta$  cells [158-161]. In contrast, IGF-1 is produced locally in the brain and does not depend on growth hormone influence as is the case of liver and other tissues [148].

Neuronal insulin receptors are different than those found in the periphery [162]. Insulin receptors are present in one of two isoforms; the IR-A isoform that lacks exon 11 that the other isoform, IR-B, expresses [163, 164]. A major functional difference between the two isoforms is

that IR-A has a higher affinity for the neurotrophic factor Insulin-like Growth Factor – 2 (IGF-II) [165] and a slightly higher affinity for insulin [166] and has also been shown to associate/dissociate with insulin quicker than IR-B [149]. Brain specific insulin receptors are mainly the IR-A isoform and as result of differential glycosylation have a lower molecular weight than their peripheral counterparts [162].

Structurally, the insulin receptor is a homodimer composed 2 $\alpha$  chains and 2 $\beta$  chains held together with disulphide bonds [167-169]. Insulin receptor binding of insulin/IGF-1 results in a conformational change that activates the catalytic tyrosine kinase activity of the  $\beta$  subunits [170]. This activation of the insulin receptor results in autophosphorylation at multiple tyrosine residues [171, 172] including tyrosine 960 in the juxtamembrane region of the  $\beta$  subunit [173, 174]. Phosphorylation at this site is a vital component of the insulin signaling cascade because it provides a binding motif for the phospho-tyrosine binding (PTB) domain of IRS [173, 174]. Once docked to the insulin receptor, IRS is phosphorylated on tyrosine residues [170].

Tyrosine phosphorylation of IRS proteins creates binding sites for Src homology 2 (SH2) domain containing proteins such as PI3K [175]. PI3K catalyzes the production of 3'phosphoinositide secondary messengers which are critical to the insulin signaling cascade. PI3K is composed of a catalytic p110 subunit and a regulatory p85 subunit that contains SH2 domains that interact with activated IRS [176]. Formation of the IRS/PI3K complex increases the catalytic activity of the p110 subunit [177].

3'phosphoinositides produced by PI3K are important signal conductors that bind to PH (pleckstrin homology) domains on proteins such as IRS [177] and Akt [178]. This interaction is needed to bring IRS and AKT proteins towards the inner layer of the plasma membrane near the juxtamembrane region of the insulin receptor [179] and in close proximity to activating kinases, respectively [180-185]. Furthermore, binding of 3'phosphoinositides is required for Akt to be competent for phosphorylation [184, 186-188].

Akt has two phosphorylation sites, Thr 308 and Ser 473, capable of inducing catalytic activity [189]. PDK1, which also depends on 3'phosphoinositides for its function, phosphorylates Akt at Thr 308 [189, 190]. While overexpression of PDK1 has been shown to activate Akt [186], optimal activation of Akt requires additional phosphorylation at Ser 473 by mTORC2 [191] which stabilizes the conformation state of Akt [192].

Akt mediates the neurotrophic effects of insulin/IGF-1, in part, by inhibiting pro-apoptotic machinery [193] and concomitantly activating anti-apoptotic proteins [194-198]. Akt's role in neurotrophic support also involves the regulation of survival transcription factors such as NF- $\kappa$ B [199] and CREB [198] as well as those involved in pro-death gene expression such as the FoxO family [200-202]. Moreover, Akt is involved in production of the neurotrophin BDNF [198], activation of proteins involved in neurite outgrowth (for review see: [203]) and regulation of the metabolic protein GSK-3 $\beta$  [204].

**Akt and Bcl-2 family members** The Bcl-2 family is a structurally related group of proteins that regulate cell death through effects on the mitochondria [205] (for review see [206, 207]). Bcl-2 members include the pro-apoptotic proteins BID, BIM, PUMA, BAD, NOXA, BAX, and BAK [205] along with anti-apoptotic mediators such as Bcl-2 and Bcl-xL [205]. Because Bcl-2 proteins

possess the ability to form heterodimers with one another [208-210], their regulation of apoptosis can be described as a balancing act in which an increase of anti-apoptotic members leads to survival while increased pro-death proteins result in apoptosis.

Mitochondrial stress incurred by ROS can lead to elevated  $Ca^{2+}$  levels in the mitochondrial matrix [211, 212] resulting in increased mitochondrial membrane permeability and release of pro-apoptotic factors such as Cytochrome c, and AIF (apoptosis inducing factor) [213]. Bcl-xL is an anti-apoptotic Bcl-2 family member that prevents  $Ca^{2+}$  induced mitochondrial permeability [214]. In the absence of insulin/IGF-1 stimulation, the survival effects of Bcl-xL are blocked as Bcl-xL is complexed with the pro-death Bcl-2 family member Bad [215-217]. Akt liberates Bcl-xL by phosphorylating Bad [195-197, 218] allowing for mitochondrial stabilization.

Mitochondrial permeability marks a critical event in the cell death cascade. Akt promotes cell survival prior to Cytochrome c release through Bcl-xL activity but has also been found to act post apoptotic factor release. When Cytochrome c is released from the mitochondria, it will associate with Apaf-1, dATP and Caspase-9 forming a structure known as the apoptosome (For review see [219]). Formation of the apoptosome activates the proteolytic activity of caspase-9 which cleaves and activates other caspases critical to the apoptotic process [220, 221]. Akt blocks apoptosome formation by phosphorylating Caspase 9 [193].

Bcl-2 is another anti-apoptotic protein under the control of Akt [222]. Bcl-2's role in cell survival is similar to that of Bcl-xL in that it maintains mitochondrial membrane integrity [223]. Mitochondrial permeability has been linked to an oxidized shift in the mitochondria [224] while Bcl-2 has been shown to promote a more reduced state [225]. Up-regulation of Bcl-2 may lead to higher cell reductive capacity [224] which is supported by the observation that Bcl-2 overexpressing cells show increased amounts of NADPH and are resistant to ROS generation [226].

The Bcl-2 promoter contains a cAMP response element site (CRE) that can enhance Bcl-2 expression by binding the transcription factor CREB. Akt is known to phosphorylate CREB which results in increased CREB binding to CBP and increased transcriptional activity [198]. Therefore, the ability of Akt to promote cell survival is mediated, in part, by influence over gene expression such as the up-regulation of Bcl-2 [227-230] and through direct protein interactions such as Bad phosphorylation resulting in Bcl-xL liberation [194-197].

**Akt and transcription factor regulation** Also under CREB transcriptional control is the neurotrophic factor BDNF [231, 232] which is essential for neuronal development, differentiation, synaptic plasticity, neuroprotection and restoration against a broad range of cellular insults [233]. BDNF has been a focus of AD research for its ability to stimulate non-amyloidogenic APP processing pathways [234, 235] in addition to protecting neuronal cultures against the cytotoxic effects of  $A\beta$  [236]. This indicates that decreased insulin signaling resulting in reduced BDNF production may be a contributing factor in AD development. In accordance, AD patients have decreased serum BDNF concentrations compared to healthy, elderly subjects [237-241] while reduced BDNF levels were associated with decreased cognitive performance in healthy individuals [242].



The transcription factor NF- $\kappa$ B is also under Akt control [199]. Like CREB, NF- $\kappa$ B plays critical roles in neuron survival [201, 243, 244] and is also involved in neurite outgrowth, myelin formation and axonal regeneration [245]. Genes for antioxidant proteins such as MnSOD [246] and Cu/ZnSOD [247] and anti-apoptotic proteins Bcl-2 and Bcl-xL are targets of NF- $\kappa$ B [248].

In its inactive form, NF- $\kappa$ B is bound to I $\kappa$ B proteins that sequester it to the cytosol (for review see [249, 250]). NF- $\kappa$ B is activated when I $\kappa$ B proteins are phosphorylated by I $\kappa$ B Kinase (IKK) complexes and targeted for degradation which allows NF- $\kappa$ B to translocate to the nucleus where it binds to regulatory DNA sequences [251]. The IKK complex consists of catalytic IKK $\alpha$  and IKK $\beta$  subunits and a regulatory IKK $\gamma$  subunit [251]. Akt facilitates NF- $\kappa$ B activation by phosphorylating IKK $\alpha$  at a critical regulatory site that promotes IKK activation [252] and subsequent I $\kappa$ B degradation.

Akt influence is not limited to only survival transcription factors but extends to pro-death modulators as well [253, 254]. The forkhead box class O (FoxO) family of transcription factors contribute to apoptosis through the induction of pro-death genes such as Fas L [201, 255, 256] and the Bcl-2 member BIM-1 [257]. Fas L facilitates apoptosis by activation of caspases [258] while BIM-1 activates the pro-apoptotic Bcl-2 family member BAX [259]. In the absence of Akt, FoxO transcription factors are transcriptionally active in the nucleus [200-202]. Akt phosphorylates FoxO family members at a conserved c-terminal sequence [253] which leads to nuclear exclusion and inhibition of transcriptional activity.

p53, another pro-death transcription factor known to be inactivated by Akt, [260] induces the expression of the pro-apoptotic Bcl-2 family member BAX. BAX proteins form oligomers that insert into the outer mitochondrial membrane which provide a passageway for Cytochrome c and other pro-apoptotic proteins to escape through [261]. Increased p53 activity leading to BAX expression has been linked to neuronal deprivation of neurotrophic factors [262].

**Akt and neurite outgrowth** Akt effects extend beyond apoptosis regulation as Akt also contributes to neurite outgrowth (for review see [203]). In hippocampal neurons Akt enhances characteristics such as dendritic length/complexity, caliber, and branching [263-267] with similar effects, excluding dendritic length, observed in dorsal root ganglia neurons [268-271]. Akt substrates implicated in neurite outgrowth include GSK-3 $\beta$  [272, 273], CREB [198], mTOR [274], peripherin [275], and  $\beta$ -catenin [276]. Akt may also work in conjunction with other pathways involved in neurite outgrowth. For example, Akt has been found to be complexed with Hsp-27 (heat shock protein) in spinal motor neurons following nerve injury [277] as well as in areas of regeneration following sciatic nerve axotomy [278].

**Akt and GSK-3 $\beta$**  Activity of the metabolic protein GSK-3 $\beta$  is also influenced by Akt. GSK-3 $\beta$  was originally identified for decreasing glycogen production through inhibition of glycogen synthase [272, 279-281]. However, GSK-3 $\beta$  is also involved in protein synthesis, cell proliferation/differentiation, microtubule dynamics, cell motility and apoptosis. Of particular interest, GSK-3 $\beta$  has also been shown to phosphorylate cytoskeletal associated tau proteins [282] which, in a diseased state, result in protein aggregates known as neurofibrillary tangles [283]. Neurofibrillary tangles have been linked to increased oxidative stress, mitochondrial dysfunction and apoptosis [284, 285] and are the most significant structural correlates of



dementia in AD [286, 287]. IGF-1 protects neurons from ischemic damage by reducing GSK-3 $\beta$  activity [288] which implies a critical role of Akt in GSK-3 $\beta$  regulation. Indeed, Akt has been shown to inhibit GSK-3 $\beta$  [204] thus demonstrating a direct role of insulin/IGF-1 signaling in the prevention of AD pathology.

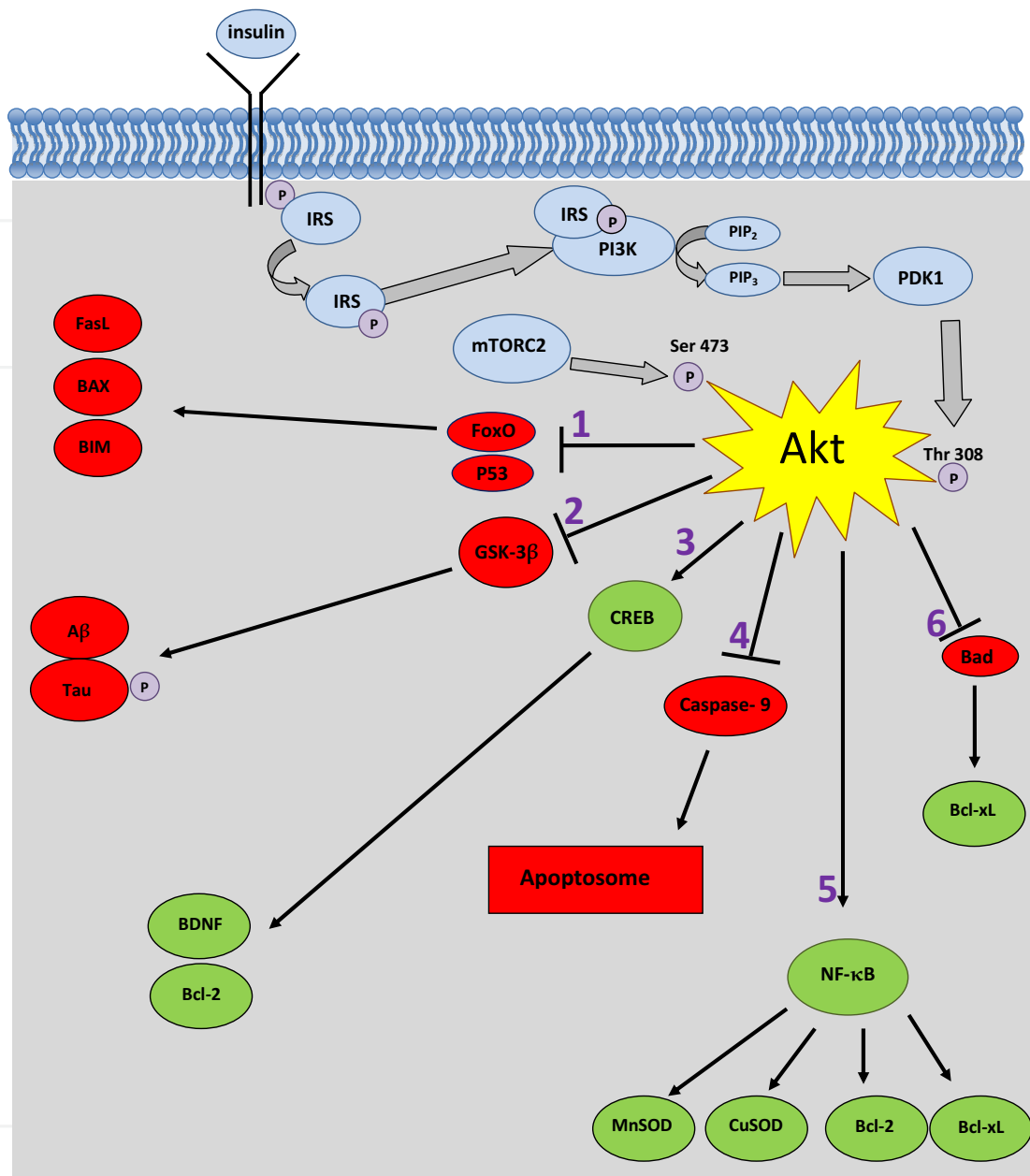
**Loss of insulin signaling** While not a cause of death on its own, loss of insulin signaling in the brain leaves neurons vulnerable to a myriad of insults. Insulin signaling is known to protect against oxidative stress, mitochondrial collapse, over-activity of GSK-3 $\beta$  leading to hyperphosphorylation of tau, activation of death promoting transcription factors and formation of apoptotic structures. Insulin also results in increased BDNF neurotrophic support as well as increased neurite outgrowth.

The mitochondrial permeability transition mediates apoptosis through the release of pro apoptotic factors. Insulin signaling maintains mitochondrial membrane integrity by increasing levels and activity of anti-apoptotic Bcl-2 family members [194-197, 227-230]. In the absence of insulin signaling, the balance of Bcl-2 proteins tips in favor of pro-apoptotic members resulting in cell death. Post mitochondrial collapse, normal insulin signaling can still prevent apoptosis by blocking formation of apoptotic complexes [193, 229] while a state of insulin resistance allows this process to continue unimpeded.

Even under normal circumstances, ROS are produced in respiratory chain reactions in the mitochondria [289]. However, if not properly managed, ROS can cause oxidative damage to proteins, lipids, and nucleic acids. Insulin supplies cells with antioxidant proteins capable of diffusing the oxidative effects of ROS by activating protective transcription factors such as NF- $\kappa$ B [246, 247, 263]. Insulin resistance not only results in reduced antioxidants but also leaves cells susceptible to ROS mediated mitochondrial collapse because of the before mentioned lack of anti-apoptotic Bcl-2 members.

The FoxO family of transcription factors is known to play a role in the cell's response to oxidative stress, however, their prolonged activation results in apoptosis [290]. Insulin signaling inactivates FoxO transcription factors through phosphorylation by Akt. Absence of insulin signaling allows FoxO members to remain in the nucleus and sustain transcription of pro-death genes [201, 255-257].

Insulin resistance is linked to structural changes in AD by overactive GSK-3 $\beta$ . Neurofibrillary tangles are a pathological hallmark of AD [283] and produced by hyperphosphorylation of tau by GSK-3 $\beta$ . Under normal insulin signaling, GSK-3 $\beta$  is inactivated by Akt. Neurofibrillary tangles are one of two significant pathological characteristics of AD the other being accumulation of A $\beta$  [291]. A $\beta$  toxicity and aggregation into plaques has devastating consequences in the brain such as synaptic disruption [292] and inhibition of LTP [293], interference of detoxifying enzymes [294], increased ROS and oxidative stress [295], increased vulnerability to calcium overload [296] and the before mentioned effects on brain vasculature. A $\beta$  also depresses insulin signaling [297] which results in further loss of neurotrophic support. Insulin signaling, on the other, hand is involved in A $\beta$  clearance [298] introducing a convoluted relationship between insulin and A $\beta$ .



**Figure 2.** Insulin receptor binding of insulin triggers a complex signaling cascade (in blue) leading to activation of the serine/threonine kinase Akt. Upon binding of insulin, insulin receptors are autophosphorylated and subsequently bind IRS proteins. IRS proteins are then phosphorylated by activated insulin receptors and complex with PI3K resulting in PI3K activation. Activated PI3K produces phospholipid secondary messengers by catalyzing the conversion of phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) to phosphatidylinositol 3,4,5-trisphosphate (PIP<sub>3</sub>). PIP<sub>3</sub> messengers activate PDK1 which phosphorylates Akt at Threonine 308. Akt is further activated by phosphorylation at Serine 473 by mammalian target of rapamycin 2 (mTORC2). Targets of activated Akt include pro-apoptotic mediators (in red) as well as pro-survival machinery (in green). Loss of insulin signaling (at sites labeled with numbers 1-6 in purple) allows FoxO and p53 transcription factors to remain active and (1) transcribe genes for pro-apoptotic proteins such as BIM, BAX and FasL. Akt inhibits the activity of GSK-3 $\beta$  that, when active, (2) causes increased amyloidogenic processing and hyperphosphorylation of tau. Other pro-apoptotic proteins inhibited by Akt include (3) caspase-9, which forms an apoptotic structure known as the apoptosome, and (6) Bad, which blocks activity of the anti-apoptotic protein Bcl-xL. Pro-survival modulators regulated by Akt include CREB and NF- $\kappa$ B. Reduction of CREB transcriptional activity as a result of a loss of insulin signaling leads to (4) decreased BDNF and Bcl-2 expression while inhibition of NF- $\kappa$ B leads to (5) reduced expression of anti-oxidants such as MnSOD and CuSOD as well as anti-apoptotic Bcl-2 family members.

## 4. Generation of A $\beta$

**Background** A $\beta$  is a small peptide 38-43 amino acids in size long believed to have a major role in neurodegeneration and pathology of AD (for review see [299]). In sporadic AD (sAD), which accounts for over 90% of AD cases, A $\beta$ 's role in pathogenesis is still under heavy investigation. The cause of familial AD (fAD), however, has been linked to 3 mutations involved in A $\beta$  processing; presenilins 1 and 2 (PS1/PS2), which are part of A $\beta$  producing complexes, and amyloid precursor protein (APP) from which A $\beta$  is derived [300]. Successive cleavages of APP by  $\beta$ - and  $\gamma$ -secretases produce toxic A $\beta$  peptides (for review see [301]) while cleavage by  $\alpha$ -secretase produces the neuroprotective product Secreted APP alpha (sAPP $\alpha$ ) [302].

While the physiological role of APP remains unknown, it has been suggested that APP plays a part in neurite outgrowth, synaptogenesis, neuronal trafficking along the axon, transmembrane signal transduction, cell adhesion and calcium metabolism, all of which still require in vivo evidence (for review see [303]). APP concentrations are elevated in the brain during the prenatal period in mice which implies a role of APP in brain development [304]. In the adult brain, APP is expressed in regions of synaptic modification [304] and has been shown to increase hippocampal neuronal response to glutamate [305].

APP belongs to a family of transmembrane proteins that includes APP-like protein 1 and 2 (APPLP1/APPLP2). All APP family members are processed in a similar fashion by  $\alpha$ ,  $\beta$ , and  $\gamma$  secretases [306-308], however the A $\beta$  domain is unique to APP. Three isoforms of APP have been identified consisting of 695, 751, or 770 amino acids which arise from alternative splicing of the same gene located on chromosome 21 [309]. APP 751 and APP 770 are expressed in most tissues and contain a 56 amino acid Kunitz Protease inhibitor (KPI) domain not found in the neuron specific 695 isoform [310, 311]. mRNA levels of the 2 KPI containing isoforms are elevated in AD brains and are associated with A $\beta$  deposition [312].

Synthesis of APP occurs in the endoplasmic reticulum where it is then transported through the golgi apparatus to the trans golgi network where the highest concentrations of APP are found in neurons [313-315]. From there, APP can be transported in secretory vesicles to the cell surface where  $\alpha$ -secretases are located, however, A $\beta$  production occurs within the trans golgi network where  $\gamma$ -secretase complexes are thought to reside [315-318].

**APP cleavage** A $\beta$  generation requires cleavage of APP by  $\beta$ -secretase which has been identified to be BACE1 [319-322]. Several studies have found that regions of the brain affected by AD have elevated BACE1 activity and levels [319, 320]. Once identified, BACE1 became a popular therapeutic target for AD treatment. However, BACE1 knockout mice have shown reduced survivability after birth and were smaller than wild-type littermates [323]. BACE1 knockouts also present with hyperactive behavior [323] and other abnormalities such as hypomyelination of peripheral nerves, reduced grip strength and elevated pain sensitivity [324].

APP cleavage by BACE1 results in two fragments: sAPP $\beta$  and Beta Carboxyl Terminal Fragment ( $\beta$ CTF) [301, 325]. sAPP $\beta$  has been identified as a ligand for Death Receptor 6 which mediates axonal pruning and neuronal death [326]. The remaining  $\beta$ CTF can be cleaved by

$\gamma$  secretase to produce  $A\beta$  [301].  $\gamma$ -secretase is a complex composed of at least 4 components: PS1 or PS2, nicastrin, anterior pharynx defective-1 (APH-1) and presenilin enhancer-2 (PEN-2) [327, 328].  $\beta$ CTF cleavage by  $\gamma$  secretase produces either  $A\beta_{40}$  or  $A\beta_{42}$  peptides [301].  $A\beta_{42}$  is the more hydrophobic and amyloidogenic of the 2 species and makes up about 10% of  $A\beta$  produced [329]. An increased  $A\beta_{42}/A\beta_{40}$  ratio has consistently been shown in fAD patients suggesting that  $A\beta_{42}$  is critical to AD pathogenesis [330, 331].

## 5. $A\beta$ and insulin resistance

**$A\beta$  depresses insulin signaling** Insulin resistance is recognized as a contributing factor in development of AD to the point that AD has been referred to as “type 3 diabetes” [4, 5]. This coincides with  $A\beta$  being a pathological hallmark of AD as  $A\beta$  contributes to insulin resistance [297].  $A\beta$  oligomers are known to impair insulin signaling in neurons [332] by competing with insulin for receptor binding sites [297] and studies have linked  $A\beta$  oligomers to decreased insulin receptor numbers [332].

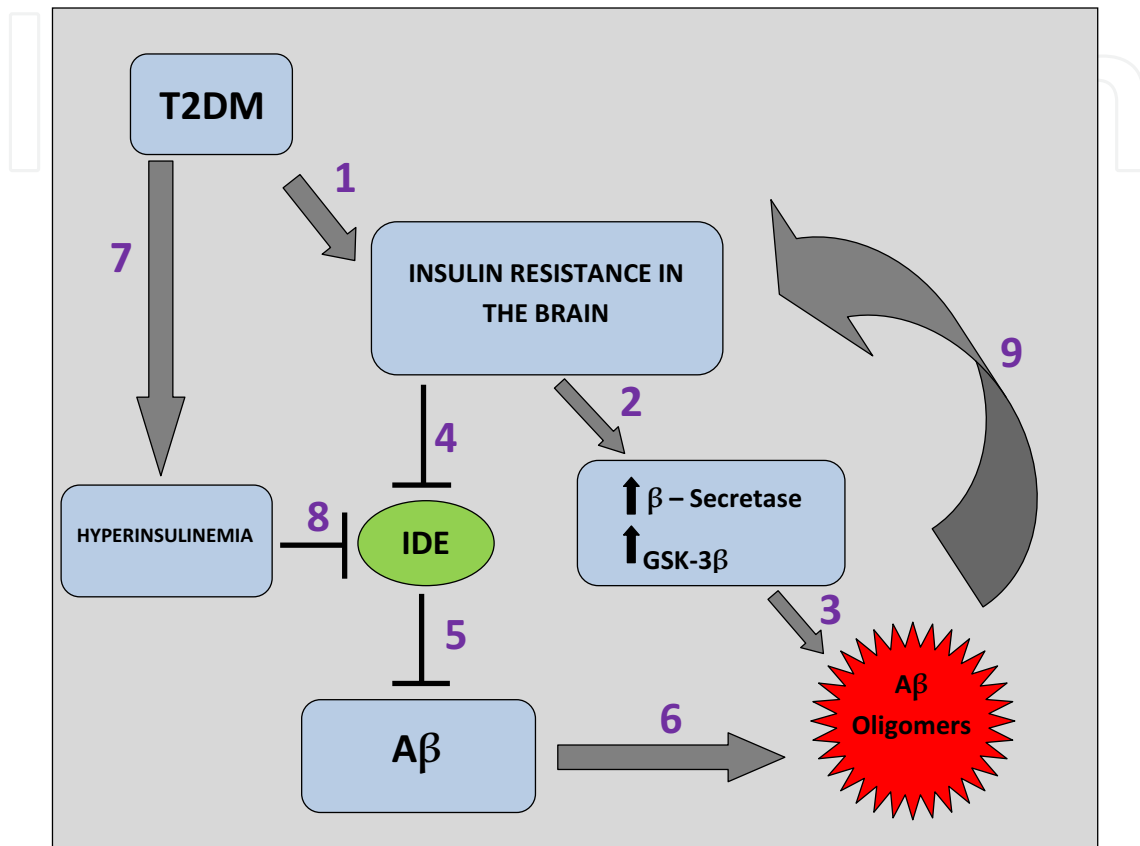
Development of insulin resistance provides neurons with a dangerous dilemma as neurons rely on insulin signaling for  $A\beta$  clearance and inhibition of amyloidogenic processing. Insulin increases  $A\beta$  trafficking from the trans golgi-network leading to secretion [333]. Secretion of  $A\beta$  may be important in preventing neurodegeneration as intraneural  $A\beta$  accumulations have been found in brain regions prone to early AD in patients with mild cognitive impairment [334] and studies done with transgenic mice indicate that intracellular  $A\beta$  accumulation is an early event of the neuropathological phenotype [335-337]. Insulin signalling protects against  $A\beta$  toxicity [298] and inhibits GSK-3 $\beta$  activity [204] which, in addition to hyperphosphorylating tau, promotes amyloidogenic APP cleavage [160, 338].

Insulin signaling pathways in the brain are complex and depend on a delicate balance of cell activity to function properly. Accumulation of  $A\beta$  perturbs this balance resulting in insulin resistance and formation of a vicious cycle as insulin signaling is no longer able to clear and regulate  $A\beta$ . As  $A\beta$  oligomers increase, insulin resistance worsens. This cycle is perpetuated by competition between insulin and  $A\beta$  as substrates for IDE.

**Insulin,  $A\beta$  and insulin degrading enzyme** IDE is responsible for insulin degradation but has also been shown to degrade  $A\beta$  peptides [339-341], a process known to be decreased in AD brains [318]. Studies have shown that increased insulin signaling can increase levels of IDE [44] which can be abolished by pharmacological inhibition of PI3K.  $A\beta$  can decrease PI3K activity, [342] and thus is able to prevent its own degradation. In cases of hyperinsulinemia, excess insulin blocks IDE binding sites which further diminishes  $A\beta$  degradation [115].

In summary,  $A\beta$  contributes to insulin resistance [297, 332] by occupying binding sites on insulin receptors [297] and is associated with decreased insulin receptor numbers in neurons [332]. Decreases in insulin signaling result in increased  $A\beta$  processing as well as activation of GSK-3 $\beta$  which promotes  $A\beta$  processing [160, 338]. Insulin signaling impairment also leads to decreased IDE, which is needed to degrade  $A\beta$  [339-341, 343]. IDE deficiencies are exacerbated

in hyperinsulinemic conditions as IDE binding sites are overloaded with excess insulin and made unavailable for A $\beta$  [115]. Lack of insulin signaling and IDE availability allows for continued accumulation of A $\beta$ , further depression of insulin signaling systems, increased neuronal vulnerability and further neurodegeneration.

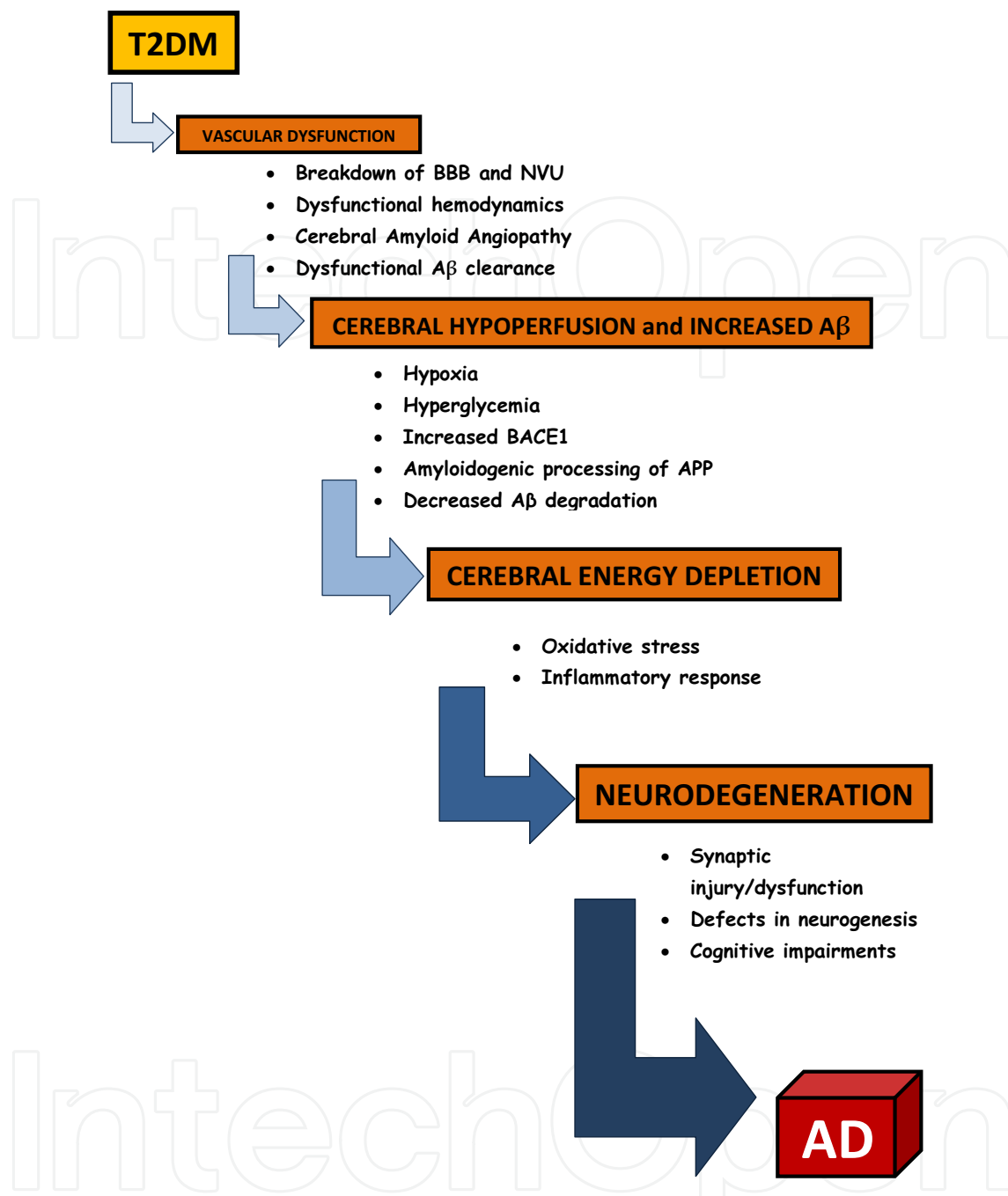


**Figure 3.** T2DM can lead to the induction of insulin resistance in the brain. (2) Reduction of insulin signaling in the brain increases the activities of GSK-3 $\beta$  and  $\beta$  secretases which (3) increase levels of toxic A $\beta$  oligomers. Furthermore, (4) insulin resistance lowers the expression of A $\beta$ -degrading IDE. (5) Reduced IDE then leads to increased A $\beta$  and (6) accumulation of A $\beta$  oligomers. T2DM also causes (7) hyperinsulinemia which exacerbates IDE deficiencies because (8) excess insulin occupies IDE binding sites rendering them unavailable for A $\beta$ . The increased amyloidogenic processing that occurs in insulin resistance combined with decreased A $\beta$  clearance by IDE results in a deleterious positive-feedback cycle as (9) A $\beta$  oligomers contribute to insulin resistance in the brain. As A $\beta$  levels continue to rise, insulin resistance worsens leading to further production of the toxic peptide.

## 6. Conclusion

By 2050 it's estimated that over 100 million people worldwide will have AD [344] causing a substantial financial burden for health care systems. In that same time span, the annual cost of treating AD is predicated to exceed \$1 trillion in the United States alone [345]. These crippling social and economical effects place increased priority for advancement of AD research.





**Figure 4.** Vascular hypothesis of AD. The vascular complications have been casually linked to the progression of AD. Vascular dysfunction resulting from type 2 diabetes results in a state of cerebral hypoperfusion, leading to significant energy depletion in the brain. Neurodegeneration results in cognitive impairments and ultimately AD.

While AD remains a disease of more questions than answers, a wide array of evidence suggests a close relationship between AD and T2DM. T2DM has been characterized as having both macrovascular and microvascular complications that result in CVD. It is the vasculature that provides the tangible pathological link between T2DM and AD. Significant data has been collected in favor of the vascular hypothesis of AD, which is founded on the idea that pre-existing CVD sets into motion pathological cascades that ultimately result in AD.

AD and T2DM also share commonality in the form of insulin resistance. Lack of insulin neurotrophic support in the brain leaves neurons defenseless against oxidative stress, A $\beta$  toxicity and apoptosis. A $\beta$  is especially dangerous to neurons because it further depresses insulin signaling and can alter levels of protective enzymes involved in its degradation such as IDE. AD is a disease that not only causes death in weakened cells but also further depresses protective mechanisms making recovery unattainable.

Because AD affects multiple structures and pathways, it is likely that successful treatment will involve a comprehensive battery of therapeutics rather than a single therapy. T2DM plays a major role in vascular abnormalities and insulin resistance which parallel AD pathologies. As a result, further exploration of the relationship between T2DM and AD may be a promising direction of future research. Moreover, preventative measures against T2DM such as proper diet and dedication to an active lifestyle may take center stage as a means of curbing the AD epidemic.

## Author details

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