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Vascular Dysfunction Associated with Type 2 Diabetes and Alzheimer's Disease: A Potential Etiological Linkage

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The endothelium performs a crucial role in maintaining vascular integrity leading to whole organ metabolic homeostasis. Endothelial dysfunction represents a key etiological factor leading to moderate to severe vasculopathies observed in both Type 2 diabetic and Alzheimer's Disease (AD) patients. Accordingly, evidence-based epidemiological factors support a compelling hypothesis stating that metabolic rundown encountered in Type 2 diabetes engenders severe cerebral vascular insufficiencies that are causally linked to long term neural degenerative processes in AD. Of mechanistic importance, Type 2 diabetes engenders an immunologically mediated chronic pro-inflammatory state involving interactive deleterious effects of leukocyte-derived cytokines and endothelial-derived chemotactic agents leading to vascular and whole organ dysfunction. The long term negative consequences of vascular pro-inflammatory processes on the integrity of CNS basal forebrain neuronal populations mediating complex cognitive functions establish a striking temporal comorbidity of AD with Type 2 diabetes. Extensive biomedical evidence supports the pivotal multi-functional role of constitutive nitric oxide (NO) production and release as a critical vasodilatory, anti-inflammatory, and anti-oxidant, mechanism within the vascular endothelium. Within this context, we currently review the functional contributions of dysregulated endothelial NO expression to the etiology and persistence of Type 2 diabetes-related and co morbid AD-related vasculopathies. Additionally, we provide up-to-date perspectives on critical areas of AD research with special reference to common NO-related etiological factors linking Type 2 diabetes to the pathogenesis of AD.

MeSH Keywords: Nitric Oxide • Diabetes Mellitus, Type 2 • Free Radicals • Endothelial Cells • Etiology • Alzheimer Disease

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Introduction: Diabetes

Diabetes mellitus (DM), one of the major leading chronic morbidities worldwide, is continually increasing with a high prevalence from 100 million in 1999 to 382 million in 2013, and to over 592 million by 2035 estimated by the International Diabetes Federation (IDF) [1,2]. Maturity-onset DM (Type 2 DM or T2DM), the most common form of diabetes, accounts for over 85% cases in all diagnosed patients, and tends to increase in children and adolescents [1]. In the United States, approximately 10% to 20% of the population older than 45 years of age were diagnosed with T2DM in 1999 [1]; by the year 2013, a total of 25.8 million Americans (some 8.3% of the population) were diabetic, of which, approximately 90–95% have T2DM [2]. Great progress has been made regarding the pathogenesis of T2DM in the past decade with particular attention on the intertwining relationship between vasopathies and T2DM.

Diabetes-associated vascular dysfunction in multiple organs has long been considered as the sequelae of the progressive disease [3], whereas some other disorders like hypertension, hypercholesterolemia, retinopathy, etc. were regarded as comorbidities of diabetes [4], and more recently, new evidence indicated that the genesis of diabetes is most likely a result of vascular dysfunction [5], and the heterogeneity property of diabetes was also highlighted [6]. Although the risk factors of T2DM have been known for many years, the vascular dysfunction-associated aspect has become a new factor, considered to be an essential contributor to the new-onset T2DM.

A well-functioning vascular system plays a pivotal role in keeping the organs healthy under both non- and genetic conditions. However, when this normal state is affected by different extrinsic or/and intrinsic factors, and then some pathologic changes occur, especially when predisposing factors exist, they work chronically to enhance the pathology. One example for this relationship is that the major four classes of drugs that are commonly used for cardiovascular risk reduction (statins, niacin, thiazide diuretics, and β-blockers) have been shown to increase the risk of new-onset diabetes by 9% to 43% [5]. The stability of vascular function largely depends on the prompt action of different mediators working precisely on the whole. Solid evidence has confirmed the importance of a functionally intact endothelium for preventing vascular dysfunction [7].

Dysfunction of multiple aspects of the endothelium has been identified in diabetes mellitus. In human retinal endothelial cells, modulation of plasminogen activator synthesis by insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF) or acidic fibroblast growth factor (AFGF) is known to be influenced by diabetes [8]. Alterations in the synthesis and release of von Willebrand factor (vWF), an important factor for efficient platelet adhesion, are common in diabetic endothelium [9]. Abnormal glycosylation of intracellular and plasma proteins occurs and affects endothelial function in diabetic patients [10,11]. Recently, cumulating evidence indicated that endothelium-dependent vasomotion was affected by DM and also contributed to the new onset DM.

In this review, we will summarize the new developments on the interrelationship between T2DM and vascular dysfunction, and provide evidence that vascular-functioning mediators are involved in the pathogenetic regulation of T2DM, and discuss complicated disorders with vascular dysfunction that are strongly associated with T2DM.

Causal Relationship Between Vascular Dysfunction and Type 2 Diabetes

It is ascertained, at least in part, that a correlation exists between vascular dysfunction and the occurrence of T2DM, but we still do not know the precise causal relationship between them. Indeed, the pathology may be initiated metabolic dysregulation, leading to poor energy production in the microenvironment. Thus, the association may have existed in the beginning of the disorder and in Alzheimer's disease (AD) as well.

Vascular dysfunction, including microvascular and macrovascular, results from DM affecting several organs (muscle, skin, heart, brain, eyes, and kidneys, et al.) [3]. The common etiology link for the different types of diabetes-associated vascular diseases is the chronic hyperglycemia that evokes pathologic responses in the vasculature, which finally cause constitutive nitric oxide (cNO) inhibition, smooth muscle cell dysfunction, overproduction of vascular endothelial growth factor (VEGF), chronic inflammation, hemodynamic dysregulation, impaired fibrinolytic ability, and enhanced platelet aggregation. In these situations, diabetes, specifically T2DM, was considered as the initiating factor for the series of vascular diseases with the contribution of common risk factors like hypertension, tobacco use, and obesity.

Although it has been confirmed that the on-going diabetes plays a crucial part in inducing subsequent vascular dysfunction through affecting endothelium [3,12], the concerns regarding the priority (i.e. which comes first – vascular dysfunction or diabetes) was raised recently. The question was derived from the evidence that statin therapy increased the risk of new onset diabetes (NOD), of which belongs to T2DM, by 9% to 21% [13,14], and this was validated by a more recent study, in which if the patients had 2–4 risk factors for NOD, atorvastatin 80 mg increased the risk of NOD by 24% compared with standard therapy [15]. From these studies, at least in part, it can be deduced that vascular dysfunction itself could initiate diabetes even though the underlying mechanisms are not yet unknown.

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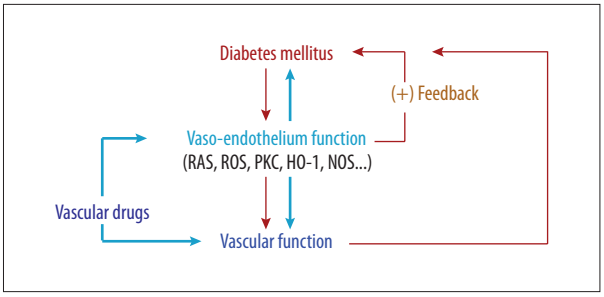

Figure 1. Interrelationship between diabetes and vascular system. Diabetes originally evokes disturbance in vaso-endothelium function that eventually changes vascular responses, which in turn form a positive feedback circuit with diabetes. In normal patients, vascular drugs can function as an initiator of new onset diabetes through mediating vascular and vaso-endothelium function. Almost simultaneously, pathologies associated with Alzheimer's Disease may emerge given the discussed commonalities since all can be linked to mitochondrial responses.

One potential mutual link between diabetes and vascular endothelium exists which determines the outcomes of the patients. In this proposed link, DM affects vascular endothelium functions through serious vaso-associated systems and pathways (see details on "Diabetes Endothelium" below), which results in changes in vascular function. Significantly, the altered endothelium and blood vessels positively are deteriorating in diabetes, i.e. a positive feedback formed. Moreover, exogenous cardiovascular medications may cause new onset diabetes through impacting endothelium and overall vascular function (Figure 1).

Dynamic Diabetic Endothelium

Endothelium is the major part of the vasculature in regulating vascular function. In the context of T2DM, chronic stimulation of hyperglycemia activates clusters of intrinsic vaso-regulating systems or pathways in vascular endothelium [16]. Understanding the dynamic underlying mechanisms is critical for understanding the diabetes-associated vascular dysfunction so that preventive strategies can be developed.

Renin-angiotensin system

The renin-angiotensin system (RAS) is linked to local regulatory mechanisms that contribute to a great number of homeostatic pathways, including cellular growth, extracellular matrix formation, and apoptosis, whereas particular concerns were raised on its role in endothelium and vascular function. Blockade of the RAS can improve visual acuity and a short-term reduction in central macular thickness in patients with refractory diabetic macular edema [17], and blockade of angiotensin II attenuates VEGF-mediated blood-retinal barrier breakdown in diabetic retinopathy [18]. Furthermore, inhibition of RAS may ameliorate islets function of diabetic rats by increasing the microvessel density in islets [19], and can increase the resistance to streptozotocin-induced diabetes, reduce inflammatory markers, and improve islet cell function [20]. Additionally, evidence accumulated to date indicates that reduction in RAS activity can prevent NOD, and the blockers are considered as the first choice drugs in hypertensive patients with diabetes to prevent the occurrence and progression of complications in diabetes [21].

Reactive oxygen species

Reactive oxygen species (ROS) are important secondary messengers for signaling pathways associated with apoptosis, proliferation, damage and inflammation. Diabetes is a condition of increased oxidative stress and requires antioxidants [22]. In diabetic condition, the delicate equilibrium between ROS production and antioxidant capability is distorted resulting in oxidative stress and further tissue injury. Vascular cells function as the major part of reactive oxygen species production, which underlies the pathogenic progression of diabetes [23]. Increased generation of reactive oxygen species from multiple enzymatic sources promotes insulin resistance, specifically at the level of the endothelium, and leads to acceleration of atherosclerosis in areas with disturbed flow patterns [24]. Based on these findings, counter regulating of ROS was studied to reverse associated vascular dysfunction. α-Melanocyte-stimulating hormone, a naturally occurring endogenous peptide hormone of the melanocortin family, can normalize oxidative stress, reduce apoptosis and ultrastructural injuries, and correct gene expression levels in early diabetic retinas [25]. Augmented superoxide dismutase 2 (SOD2) ubiquitination leads to the increase in mitochondrial ROS concentration in coronary endothelium from T2DM mice and attenuates coronary vascular relaxation [26]. Moreover, direct anti-ROS treatment can prevent exacerbation of inflammation and insulin resistance that alleviates diabetic pathologies [27]. Amelioration and/or prevention of vascular endothelial and contractile dysfunction by doxycycline is strongly associated with a clear reduction in oxidative stress markers of diabetes, suggesting a therapeutic intervention for amelioration and/or prevention of vascular disorders in diabetic subjects [28].

Protein kinase C pathway

The regulatory enzyme protein kinase C (PKC) is known to play a key role in vascular tone regulation in physiological and pathological conditions[30]. The association of protein kinase C (PKC) with vascular alterations such as increases in permeability, contractility, extracellular matrix synthesis, cell growth and apoptosis, angiogenesis, leukocyte adhesion, and cytokine activation and inhibition has been confirmed [30]. These

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perturbations in vascular cell homeostasis, caused by different PKC isoforms (PKC-α, -β1/2, and PKC-δ), are linked to the development of pathologies affecting large vessel (atherosclerosis, cardiomyopathy) and small vessel (retinopathy, nephropathy and neuropathy) complications in the context of diabetes [29]. Activated PKC in diabetes increases VEGF expression and activates NADPH oxidases leading to raised ROS production. In addition, PKC in DM is involved in enhancement of vascular contractility in an endothelium-independent manner by inactivation of K+ channels and Ca2+ sensitization of myofilaments in vascular smooth muscle cells [30]. PKC-β phosphorylates occludin regulating tight junction trafficking in VEGF-induced permeability *in vivo* [31]. Silencing of the PKC-δ gene expression using siRNAs led to restoration of vasodilator potential in rats with diabetes [32]. PKC inhibition ameliorates functional endothelial insulin resistance and smooth muscle cell hypersensitivity to insulin, but does not restore acetylcholine-activated endothelium-dependent vasodilation in diabetic rats [33]. The function of PKC pathway in diabetic context is intricate. How to reach an ideal function of PKC in the regulation of vascular function needs in-depth investigation.

Heme oxygnase-1/carbon monoxide

The intracellular levels of carbon monoxide (CO) can increase under stressful conditions following the induction of heme oxygnase-1 (HO-1), a ubiquitous enzyme responsible for the catabolism of heme. Although CO does not contain free electrons, it may still be involved in oxidative stress, and Heme oxygenase-1 (HO-1) was found to be a key defense mechanism against oxidative injury [34]. HO-1 possesses a protective effect against aortic endothelial dysfunction in the insulin resistance (IR) state by inducing antioxidation and promoting regulative effect of vasoactive substances [35]. Induction of HO-1 with hemin ameliorates the abnormality of endothelium-dependent vascular relaxation in T2D rats through suppressing reactive oxygen species production and inhibiting COX-2 upregulation induced by diabetes mellitus [36], and ameliorating exaggerated vascular contractility by reducing TNF-α and aortic ROS levels [37] and pulmonary artery by involving a reduction in inducible NO synthase-derived NO production [38].

Further studies found the enhancement of the HO system facilitates insulin sensitivity and glucose metabolism in diabetic animals [39,40]. HO-1-induced increase in eNOS and decrease in iNOS are potentially contributing to restoration of vascular responses in diabetic rats [41], and HO-1 up regulation in diabetic rats brings about an increase in serum bilirubin, a reduction in O*-2 production, and a decrease in endothelial cell sloughing [42], and amelioration in postprandial and fasting hyperglycemia in T2DM rats [43]. Therefore, attention is being paid to the use of selective HO inhibitors that were considered very important tools to clarify the role of the HO system and the mechanisms underlying its physiological effects and pathological involvement; due to the inducible nature of HO-1, selective inhibition of HO-1 isoforms is generally preferable. Notably, HO-1 inhibitors may be also beneficial in therapeutic applications.

Nitric oxide system

The vascular endothelium is a multifunctional organ and is critically involved in modulating vascular tone and structure. Nitric oxide (NO) is a short-lived gaseous signaling molecule in mammals. The regulation of NO bioavailability is critical to maintain blood vessel function. Endothelium-derived NO has been demonstrated to mediate many important endothelial properties [44]. NO inhibits the adhesion and aggregation of platelets and the release of their contained growth factors, the chemotaxis and activation of mononuclear leukocytes, the expression of leukocyte adhesion molecules by activated vascular endothelium, and the migration and proliferation of smooth muscle cells. It decreases endothelial permeability for macromolecules and lipoproteins [44–46]. In addition, NO is essential for the maintenance of basal vascular tone and its regulation in response to various physiologic and pathologic stimuli [47,48]. Therefore it has been surmised that impaired activity of NO diminishes the resistance of the vascular wall to disease and disrupts vascular homeostasis.

Initially, studies in animals were performed which found both conduit and resistance arteries of chemically induced diabetes to have attenuated endothelium-dependent relaxation [49,50]. Subsequently, evidence of impaired endothelium-mediated smooth muscle relaxation in humans was discovered in the penile corpora cavernosa [51]. To date, numerous studies have confirmed that endothelium-dependent relaxation is impaired in DM. Early reports have examined NO activity in diabetic endothelium, but contradicting results have emerged [52–56]. One possible reason was that all of these studies have used indirect methods in an attempt to determine NO's presence and thus its role. As a result, no conclusive direct evidence was reached on the levels of NO release in diabetic endothelium.

One indirect method is the administration of NO trapping agents, which were used to assess the basal NO activity in the rat aorta [57,58]. These scavengers bind and eliminate NO after its release without altering NOS activity. Analysis of tension in rings pre-contracted with phenylephrine revealed that the application of NO trapping agents produced an additional increment in tension that was greater in the control than the diabetic rings. These findings were thought to suggest that the basal NO activity in diabetics is less than that in non-diabetics. Another indirect means used in assessing information regarding potential deficits in NO synthesis is the measurements of cGMP in vascular tissue [59]. Decreases in acetylcholine-stimulated cGMP

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production were observed in multiple diabetic animal models. No apparent intrinsic change in either guanylate cyclase or phosphodiesterase activity of the vascular smooth muscle was found to account for defective cGMP production in these blood vessels. Therefore, the conclusion was suggested that the basal NO bioactivity in experimental diabetes was decreased. Unfortunately, all of these investigations in both animal models and human studies make the assumption that endothelium-dependent relaxation in both control and diabetic blood vessels is exclusively mediated via NO to reach their conclusions.

Beside these developments, we first, in 2002, reported on the NO stimulated cNOS activation by morphine in diabetic human saphenous vein [7]. In this early study [7], we first employed the real-time NO release using an amperometric probe, real-time technology, to conclusively provide information on NO levels, from which we found that diabetic endothelium exhibits a diminished basal NO release in comparison to non-diabetic controls. Through the application of a NO synthase inhibitor, further information on the actual basal level of NO release was obtained. Diabetic endothelium showed no decrease in its basal release level. We believed this signifies that the actual level of NO release is negligible. By exposing the tissues to superoxide dismutase (SOD) we confirmed that the depressed levels of released NO seen were not the result of extracellular NO scavengers. Unfortunately, though we could not exclude the possibility of intracellular scavengers. Lastly, we evaluated the capacity of diabetic endothelium to produce NO when stimulated by an agonist – morphine sulfate. The results obtained were consistent with the premise that the previous findings support, NO metabolism is impaired in DM. A lower peak level and shorter duration of stimulated release, as well as a decreased expression of mu opiate receptors was seen in diabetic compared to non-diabetic tissue.

In considering the consequences of these findings in DM, a negligible basal level and a diminished capacity for stimulated endothelial NO release, some of the possible potentially pathologic resultant alterations in cellular metabolism should be mentioned. Firstly, NO has been shown to have antioxidant actions [59–62]. This scavenging property gives NO a major intracellular and extracellular action against oxidative stress [61,63–70]. Emerging evidence attested the role of endothelial NO in diabetic vascular pathologies. First, endogenous NO suppresses vascular inflammation, via inhibition of p47(phox) expression, leading to attenuation of NADPH oxidase-dependent superoxide production [71]. However, selective endothelial insulin resistance itself is sufficient to induce a reduction in NO bioavailability and endothelial dysfunction that is secondary to increased generation of reactive oxygen species [72]. Third, diabetes produces a cascade of events involving production of reactive oxygen species from the NADPH oxidase leading to oxidation of BH4 and uncoupling of NOS, which promotes the oxidative inactivation of NO with subsequent formation of peroxynitrite [73]. As thus, in the absence of NO, reactive chemical species may occur and cause tissue damage [7].

Heme proteins (e.g., hemoglobin, cytochromes, etc.) reacting with H_2O_2 results in ferryl cation (FE^{4+}=O), a toxic substance [74]. However, once in contact with NO, this compound is reduced (FE^{3+} + NO_2^-)[61,75], suggesting the antioxidant action of NO. NO also has the potential to diminish the formation of $OH^{\bullet}$, demonstrating once more an antioxidant action [60,76]. Here again we note that in the absence of NO, as potentially occurring in diabetes, these reactive chemical species may cause the tissue damage associated with diabetes's pathological progression.

Meanwhile, NO interacts with heme proteins to activate soluble guanylyl cyclase and cyclooxygenase and also has the ability to inhibit lipoxygenase [77–80]. Inducible nitric oxide synthase (iNOS) driven NO production regulates expression of iNOS, suppresses cyclooxygenase-2 (COX-2) levels, and maintains Hemeoxygenase-1 (HO-1) levels in nephritic animals [81]. However, in the context of pouchitis, another type of inflammatory disease, the increased COX-2 and NOS were not associated with HO-1 induction [82]. A more recent study showed that HO-1 regulates lipopolysaccharide (LPS) and nicotine induced the production of NO and PGE2 through affecting the expression of iNOS and COX-2 in human periodontal ligament cells [83]. These data suggest, at least in part, that altered NO functions through connecting with COX and HO-1 systems, which is involved in the mediation of subsequent inflammatory processes. One of the mechanisms is the altered formation and release of the induced substances secondary to the impaired NO levels could adversely impact the important regulation of the pro-inflammatory processes.

Additionally, NO is an inhibitor of cytochrome c oxidase, the terminal enzyme in the electron transport chain, in a manner that is reversible and competitive with oxygen, by reacting with both the two-electron and single-electron reduced active sites [84–93]. Diminished NO levels could alter the modulation of oxygen utilization, in a similar manner to how the inhibition of c-NOS derived NO increases oxygen consumption in many animal species [94–98]. Besides, the endogenously released endothelial NO, either basal or stimulated, can modulate oxygen consumption both throughout the thickness of conductance vessels and in the microcirculation, which may be implicated in the origin of vascular pathologies such as atherosclerosis [99]. Moreover, inflammatory mediators such as IL-1β hampers glucose-stimulated insulin secretion (GSIS) in Cohen diabetic rat islets through mitochondrial cytochrome c oxidase inhibition by NO. Reduced islet COX activity renders vulnerability to GSIS inhibition on high-sucrose, low-copper-diet (HSD) through inducing the expression of iNOS and nitric

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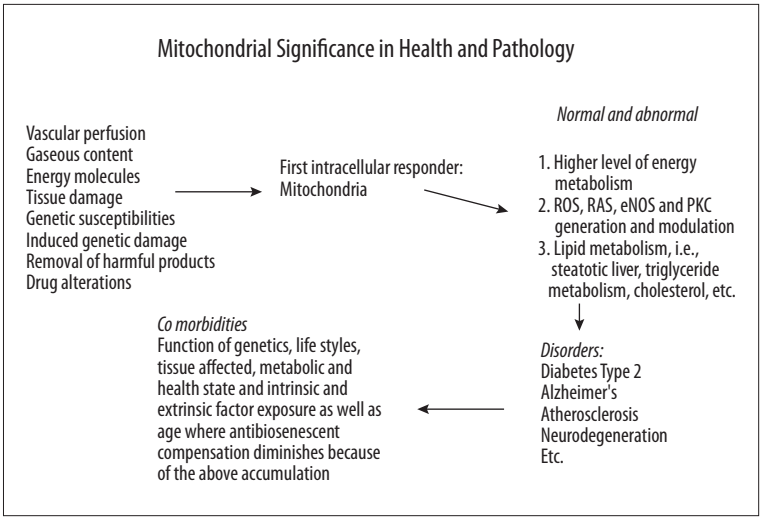

Figure 2. Illustration of the interconnectivity of health and pathology and their dependence, in part, on mitochondrial associated processes. Given the cells. Michochondria's position in its hierarchy of immediate response its significance is highlighted. It's significance is also enhanced by the number of processes that can be associated with it that can "dampen" the immediate/acute effect/stimulus of most abnormalities, making it prone to chronic debilitation. This hypothesis is further highlighted by the fact that each condition has a co morbidity that is related to a prime pathology. Thus co morbidities represent the scope of the disorder while documenting other components, which can be considered holistically as a medical target.

oxide-mediated COX inhibition [100] (Figure 2). Intriguingly, the blunted endothelial NO in patients suffering from insulin resistance T2DM played an interaction between endothelial insulin sensitivity, of which the increased insulin signaling in endothelium increases the generation of superoxide anion via activation of NOX2 NADPH oxidase and reduced NO production in response to insulin due to increased endothelial proline-rich tyrosine kinase (PYK2) activity leading to a pro-atherosclerotic state [101].

Therefore, we surmise that basal NO levels promote the health of the endothelium by limiting its immune and vascular activating potential, i.e., decreasing the appearance of an uncalled for pro-inflammatory response [7,44]. Thus, in diabetic individuals, the decrease in the capacity for this vital action leads to both enhanced vascular and immune activity, as noted by increased platelet-derived plaque formation for example [44]. The fact that stimulated NO levels are significantly diminished, but still exist, denotes the progressive nature of the vascular pathology associated with diabetes. The remaining capacity for stimulated NO release may help down-regulate mediators in the vascular and immune tissues [44]. However, since it is not continuous and diminished in the diabetic, its effect is probably only partial, allowing for a progressive decrease in the ability to down-regulate these tissues over the long term. Certainly, this may not be the only explanation for the vascular abnormalities found in diabetes mellitus given the complexity of the pathological processes, however, we do believe that this impaired NO metabolism plays a significant role.

NO and superoxide free radicals in diabetes

Additionally, NO is emerging as a central regulator of energy metabolism and body composition. In considering the consequence of a general lack of NO in severe diabetics on the cellular level, we surmise an alteration of endothelial cellular metabolism. NO can interact with oxygen, metals and other free radicals [44,102,103]. NO can form peroxynitrite (ONOO–) and dinitrogen trioxide (N_2O_3), following an interaction with the superoxide radical (O2–) and oxygen, respectively [61,104]. NO and ONOO– have inhibitory effects on purified cytochrome b5 reductase (Cb5R), a pleiotropic flavoprotein that catalyzes multiple one-electron reduction reactions with various redox partners, providing the basis for a feedback cellular protection mechanism through modulation of excessive extramitochondrial superoxide anion production by Cb5R [105]. In this regard, NO's actions are directly felt when its level is low and of short duration, occurring under physiological conditions [61]. For example, NO interaction with the heme proteins represents the activation of soluble guanylyl cyclase (sGC) and/or COX [77–79]. The sGC-cGMP signaling is one of the major pathways of NO-related vascular function mediation [106]. NO-associated COX activation is of importance in the regulation of a pro-inflammatory process [79,107]. Additionally, at low NO concentrations it modulates the redox form of COX, converting the ferrous iron to the ferric active form, acting also as a scavenger of superoxide [61]. Meanwhile, NO functions as a weak ligand to ferric heme, which, at the same time, forms a strong Fe-NO bond to regulate the reactivities of ferrous and ferric heme-nitrosyls [108]. NO also has the ability to inhibit lipoxygenase [80,109]. It can also reversibly inhibit the heme moiety of cytochrome P-450, preventing the binding of oxygen to the catalytic sites [110,111]. However, a peculiar P450, P450nor, can receive electrons directly from NADH for the reduction of NO [112]. Interestingly, at low NO levels H_2O_2 can be consumed to yield HNO_2 [61,113], suggesting that H_2O_2 might serve to control NO levels that contribute to the development of vascular diseases [61,114]. In this regard, if NO were absent, H_2O_2 may generate tissue damage and energy metabolism may proceed impaired as appears to occur in diabetes [115]. A more

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interesting finding is that endothelium-derived hyperpolarizing factor (EDHF) compensates for diminished NO-dependent dilation in IL-6-induced endothelial dysfunction by the activation of H_2O_2 or a K+ channel in T2DM, suggesting that the interaction between NO and H_2O_2 is not as simple as figuring out which depends upon which [116]. We suppose that the superoxide products in diabetic endothelium confound each other to induce vascular pathologies.

Furthermore, mitochondria represent a NO target since NO is an inhibitor of cytochrome oxidase of the electron transport process [84–89,117], suggesting again a NO role in modulating oxygen utilization [85,118]. The inhibition of cNOS-derived NO increases oxygen consumption in many animal species [94–98]. This last fact is critical to our NO hypothesis concerning diabetes (see earlier discussion). Furthermore, a NOS isoform, mtNOS, is present in mitochondria [84,119–121] supporting an important modulatory function as well.

Alzheimer's Disease and Type 2 Diabetes

Chronic vascular diseases are the major complications that account for over 90% of mortality from diabetes. Alzheimer's disease (AD), which affects 36 million people worldwide, is generally considered as an age-related degenerative disease. However, the actual etiology of AD is yet unknown. Emerging evidence indicates that the pathogenesis of AD is attributable to the chronic vascular pathologies [122].

Vasculopathy and Alzheimer's disease

Our previous work disclosed a causal relationship between vascular pathologies and AD [122–133], and we evidenced that AD may be a vascular disorder with neurodegenerative consequences rather than a neurodegenerative disorder with vascular consequences. Based on this proposal, two factors are needed to be present for AD to develop: 1) advanced ageing, 2) presence of a condition that lowers cerebral perfusion, such as a vascular-risk factor. The first factor introduces a normal but potentially insidious process that lowers cerebral blood flow in inverse relation to increased ageing; the second factor adds a crucial burden which further lowers brain perfusion and places vulnerable neurons in a state of high energy compromise leading to a cascade of neuronal metabolic turmoil. Convergence of these two factors, will culminate in a Critically Attained Threshold of Cerebral Hypoperfusion (CATCH) [122–133]. CATCH is a hemodynamic microcirculatory insufficiency that will destabilize neurons, synapses, neurotransmission and cognitive function, creating a neurodegenerative state characterized by the formation of senile plaques, neurofibrillary tangles, amyloid angiopathy and in some cases, Lewy bodies. Since any of a considerable number of vascular-related conditions must be present in the ageing individual for cognition to be disturbed, CATCH identifies an important aspect of the heterogenic disease profile assumed to be present in the AD syndrome [122].

It is proposed that CATCH initiates AD by distorting regional brain capillary structure, involving endothelial cell shape changes and impairment of NO release, which affects signaling between the immune, cardiovascular and nervous systems. Evidence indicates that in many tissues there is a basal level of NO being produced and that the actions of several signaling molecules may initiate increases or decreases in basal NO levels [44]. Moreover, these temporary alterations in basal NO levels exert inhibitory cellular actions, via cellular conformational changes. Findings indicate that 1) constitutive NO is responsible for a basal or "tonal" level of NO; 2) this NO keeps particular types of cells in a state of inhibition and 3) activation of these cells occurs through disinhibition. Consequently, tissues not maintaining a basal NO level are more prone to excitatory, immune, vascular and neural influences. Under such circumstances, these tissues cannot be down-regulated to normal basal levels, thus prolonging their excitatory state [44]. Thus, the clinical convergence of advanced ageing in the presence of a chronic, pre-morbid vascular risk factor, can, in time, contribute to an endotheliopathy involving basal NO deficit, to the degree where regional metabolic dysfunction leads to cognitive meltdown and to progressive neurodegeneration characteristic of AD [122].

The conventional view proposes that AD precedes vascular dysfunction. In this regard, an increase in the concentration of amyloid beta (Aβ) above its normal physiological level in the circulation results in decreased NO production and vessel sensitivity to endothelium-dependent vasodilation, that could lead to constricted blood vessels and ischemia in the surrounding tissue [134]. Furthermore, increases in Aβ lead to cell death and decreased maximum vasodilator response of cerebral vessels in the context of AD [134]. Nonetheless, this conception has been challenged by our previous compelling evidence discussed above, and along with our evidenced findings, other supportive data emerged from peer institutes upon the vascular originality of AD's pathogenesis. A number of review articles have provided an excellent view on the vascular contribution to the genesis of AD by focusing on the role of chronic hypoperfusion in triggering mitochondrial dysfunction in vascular cells, which, in turn, enhances the production of ROS and reactive nitrogen species (RNS) that were driven by NOS [122,135–138]. Therefore, NO-associated oxidative responses of the vascular system were considered as an initiator of brain lesions during the development of AD [122,139]. Further, NO production is IFN-γ dependent both in cognitive impairment and Alzheimer's patients, and the high levels of NO, probably iNOS driven since the condition is chronic, are

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associated with an elevation of TNF-α levels in severe stages of AD [140], suggesting the existence of a causal interaction between NO and inflammation that underlies the severity of AD and cognition impairment. Meanwhile, the causal relationship among NO, mitochondrial dysfunction, oxidative stress, and AD has been highlighted as a therapeutic target [122] (Figure 2). Widespread cerebral amyloid angiopathy and arteriosclerosis/lipohyalinosis are associated with the development of cognitive deficits in AD and a combination of them may contribute to neurodegeneration in AD, suggesting that small vessel disease due to arteriosclerosis and fibrolipohyalinosis is a potential target for the treatment of AD [141]. Elimination of the imbalance seen in energy production and restoration of the normal cellular function, make the antioxidants powerful alternates for treating vascular pathologies as well as neurodegenerative diseases [142].

Alzheimer's Disease and Diabetes Type 2 Commonalities

Strong evidence demonstrates that AD shares characteristics and possible origins with both cardiovascular disorders and diabetes [44,122,143]. Given the discussion in the earlier reports noted, which emphasized energy metabolism due to hypoperfusion, it is not difficult to speculate that DM may be a major component in the pathogenesis of AD as reflected in the associated vasculopathy, resulting in damaging the microvascular environment. In this regard, it has been suggested that DM is a risk factor for AD [44,122,144,145]. Thus, the progression of events includes hyperglycemia leading to diabetic vasculopathy via glyceraldehyde-derived AGEs Glycer-AGE. Moreover, in human AD brain, Glycer-AGE is distributed in the cytosol of neurons in the hippocampus [145].

Furthermore, hyperglycemia-induced mitochondrial dysfunction and oxidative stress have been associated with Aβ induced pro-inflammatory responses [44,122,146], which is expected, given the damage to the vascular conduit of glucose utilization. Interestingly, high glucose and Aβ1-40 reduced cell and enhanced mitochondrial $O2^{\bullet-}$ and H_2O_2 production. This situation creates an environment promoting a higher susceptibility to the deleterious actions of Aβ-40 [146] (Figure2). Semicarbazide-sensitive amine oxidase/vascular adhesion protein-1 (SSAO/VAP-1) also are implicated in AD and DM [147]. These findings support the hypothesis that DM predisposes to cerebrovascular alterations, cognitive decline, and development of AD [148]. In mice, cultured endothelial cells exposed to both glucose and Aβ generate oxygen reactive products and glycation entities, which also have been associated with cognitive decline [149]. Thus, glucose associated presence and regulation are coupled to this pathological process and its alteration may represent the first step in this deteriorating energy pathway in both DM and AD.

Recently, Grammas et al. [143] proposed that in AD animal models, the cerebrovasculature is activated and overexpresses Aβ. Importantly, we proposed a similar vascular theory in 2000 (see previous section) [44,122,144]. We demonstrated that hypoperfusion of brain areas caused endothelial cells to loosen their juxtaposed borders, creating "gaps" whereby greater brain access occurred, i.e., allowing Aβ and immunocyte entry. Given Aβ immunostimulant activities a proinflammatory reaction would go from acute to chronic over time due to the "gaps" [122]. In part, this inflammatory state was, we surmise, initiated by a dysfunctional nitric oxide release from compromised endothelia, which constitutively stabilizes endothelia and immune cells [44,122]. Supporting this concept is the fact that rat hippocampal neurons exposed to CATCH exhibit an altered nitric oxide release [150]. In summary, an impaired microvasculature is present in both DM and AD as well as vascular dementia [44,122,144]. Additionally, it would appear that the vasculopathy emerges early on, remaining below "detection", leading to the neurodegenerative processes associated with AD.

Hayden et al. [151] proposes that cognitive decline in AD results from a combination of factors some of which have long been associated with the disorder (aging, genetic, and lifestyle), which result in multiple injurious metabolic and immunologic toxicities such as dysfunctional immune responses, oxidative stress, inflammation, insulin resistance, and dysglycemia (systemically and in the brain). These converging abnormalities may lead to endothelial blood-brain barrier tight junction/adherens junction complex remodeling and microglia activation, which may result in neurodegeneration, impaired cognition, and dementia. Indeed, on an individual basis, variations in susceptibility to these alterations probably exist, leading to the variations in the expression of characteristics associated with the disorders. The fact that similar patterns of vascular anatomy and functional dysfunction are quite similar in DM and AD, suggests that intrinsic susceptibility to both or either of these disorders also exists.

Conclusions

The early development of cardiovascular complications, including accelerated atherosclerosis and microangiopathy, which occurs with T2DM, is responsible for a high morbidity and mortality. Endothelial dysfunction plays an essential role in the initiation of cellular events, evolving into the development of vascular complications in diabetes. Diminished production and function of endothelium-derived NO and corresponding factors, in combination with the overproduction of pro-inflammatory mediators and vasoconstrictors, eventually result in endothelial dysfunction, which finally elevates vascular tone that culminates in changes to micro- and macro-vascular dysfunction. The diabetic context-associated NO imbalance and the subsequent cascades of free radicals underlie the vascular dysfunction,

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which has been considered as the initiator of T2DM. In considering the potential commonalities of DM and AD, i.e. the NO-related oxidative alterations in vascular endothelium, new concerns have arisen on the preceded occurrence of DM and AD. The NO-oxidative links underlying vascular dysfunction appears to contributes to the genesis and progression of T2DM. Therapeutics targeting of ROS using antioxidants, inhibitors of the RAS, mediators increasing eNOS activity, or methods affecting vaso-function-related signaling pathways might assist in reversing the endothelial dysfunction that has been proposed. This strategy may aid in reducing the related vascular morbidity and mortality in diabetes, AD and possibly atherosclerosis.

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Conflict of interests

None.

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