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Age-related diseases as vicious cycles

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Age-related diseases (ARDs) are the leading cause of death worldwide, and contribute to 90% of mortality in developed countries. Interestingly, the mortality rates of individual ARDs increase exponentially with age. Processes described by the exponential growth function typically involve a branching chain reaction or, more generally, a positive feedback loop. Here I propose that each ARD is mediated by one or several positive feedback loops (vicious cycles). I then identify critical vicious cycles in five major ARDs: atherosclerosis, hypertension, diabetes, Alzheimer's and Parkinson's. I also propose that the progression of ARDs can be halted by selectively interrupting the vicious cycles and suggest the most promising targets. An evolutionary perspective is also offered.

Keywords: aging, age-related disease, mortality rate, positive feedback loop, vicious cycle

Nationwide mortality and disease incidence statistics are perhaps the most powerful and least biased datasets on human diseases that we currently have. These data are derived from humans living in the complex environment and developing diseases naturally, and not from distantly related animals contained in laboratory conditions under disease-inducing regimens. Whilst clinical studies share the same advantages, the number of human subjects is orders of magnitude lower, and a bias related to study design is always present.

By studying the incidence statistics of 20 most prevalent cancer types in relation to patients' age, I have previously shown that it closely follows the probability density function of the Erlang distribution[1]. The Erlang distribution describes the probability of several independent random events occurring by the given time, but not earlier or later. This fits well with the widely accepted multiple-hit hypothesis of carcinogenesis, which states that cancers arise after several successive events[2-4]. Whilst no consensus has been reached on the nature of these events, driver mutations are the most probable candidate. Such mutations confer the growth advantage, apoptosis resistance or other oncogenic properties to the cell, as opposed to inconsequential passenger mutations[5]. Overall, these results suggest that cancer is essentially a random event and not the true gradually developing ARD.

I have then decided to use disease statistics to elucidate the underlying nature of five major ARDs: atherosclerosis, hypertension, diabetes, Alzheimer's and Parkinson's. As large-scale incidence data for these diseases is not readily available, I have instead evaluated the age distribution of mortality (see Methods for details). Unlike incidence rates, mortality rates represent the hazard function derived from the probability density function. They are usually approximated by the hazard function of the Gompertz distribution, which is essentially the exponential function. However, the exponential function does not have an upper limit, whereas the mortality rate does (100,000 deaths per 100,000 people), so it cannot be the mathematically correct choice. Instead, the logistic function, which initially behaves like the exponential but then asymptotically approaches the upper limit, appears more appropriate and indeed provides an excellent fit to the data (Figure 1).

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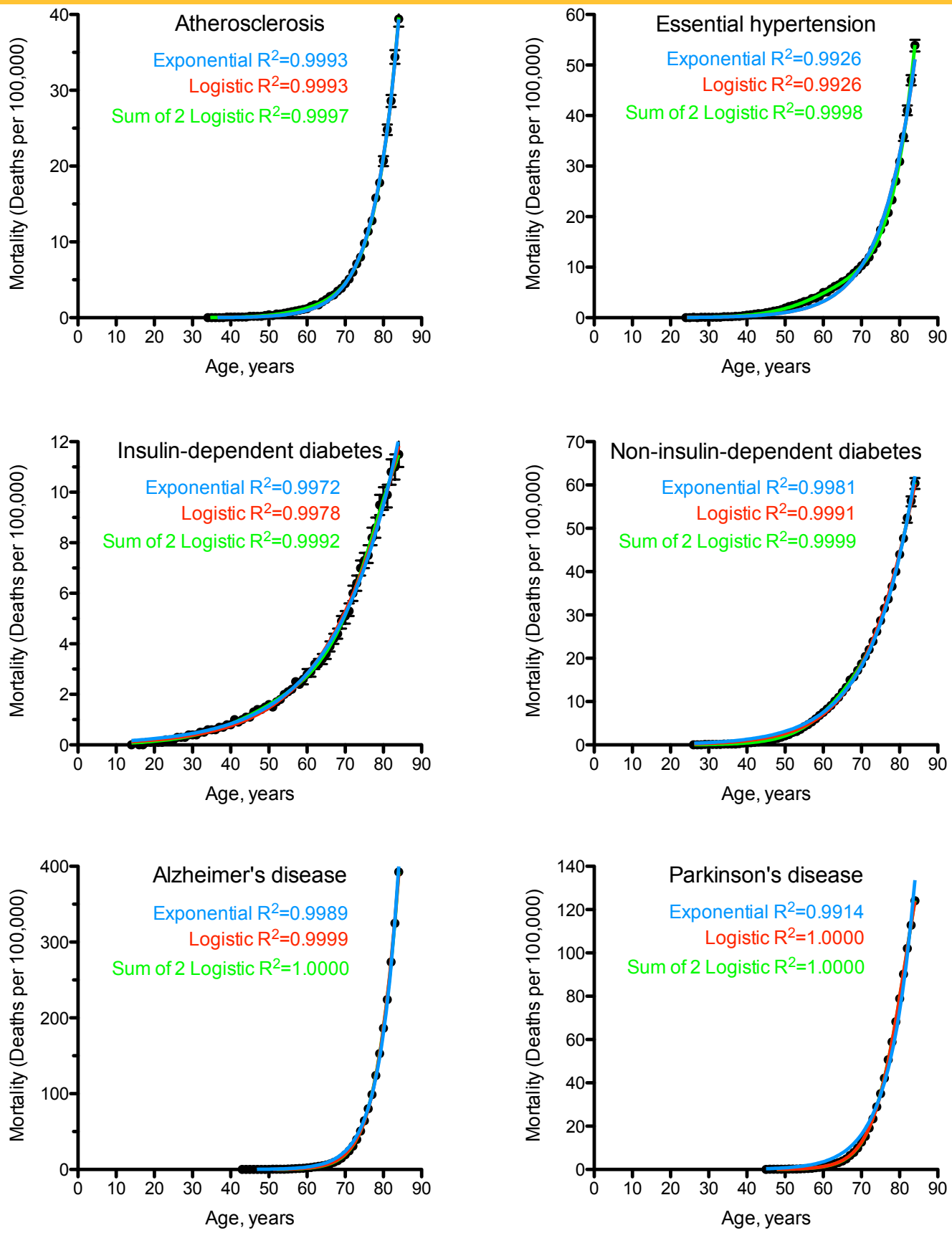

Figure 1. The logistic function is preferable over the exponential function (Gompertz hazard rate) for the approximation of the age distributions of mortality rates for age-related diseases. Dots indicate actual data for one-year age intervals. Curves indicate the exponential (blue), logistic (red) and the sum of two logistic (green) functions fit to the data. A simpler model is plotted on top of a more complex model (exponential > logistic > sum of 2 logistic).

It can be seen that the exponential function provides a reasonable approximation for mortality from atherosclerosis, diabetes and Alzheimer's, but is inadequate for mortality from essential hypertension and Parkinson's. The slightly more complex but mathematically correct logistic function provides the fits that are at least as good as for the exponential function, and in addition provides the perfect fit for Parkinson's disease mortality. Finally, the sum of two logistic functions is required for the adequate fit to mortality from essential hypertension. This may indicate that essential hypertension is a heterogeneous disease composed of two major subtypes with different mortality kinetics. Indeed, essential hypertension is defined as hypertension with an unknown cause. It has to be noted that hazard functions of common statistical distributions, including Weibull and gamma, as well as the sum of two exponential functions, failed to provide fits as good as logistic function.

As mentioned above, the logistic function describes exponential growth that slows down when some limiting factors start to play a role. Processes that exhibit an exponential growth behavior are common in natural and artificial systems. They include, but are not limited to, nuclear chain reactions, exothermal heat-accelerated chemical reactions, crystallization of water into ice, avalanches, growth of bacteria, growth of prey population after removal of predators, viral epidemics, as well as the acoustic feedback in microphone-amplifier-loudspeaker systems. All these processes involve either a branching chain reaction or, more generally, a positive feedback loop. In lay terms, they can be described as "A produces more of B, and B produces more of A", with possible intermediates C, D, E, F, etc., or in the simplest case, "A produces more of A".

The well-known property of systems containing positive feedback loops is the progressive amplification of initially small disturbances that leads to system instability and, eventually, destruction, if no negative feedback loops are in place. The vivid examples include nuclear and chemical explosions. I propose that **age-related diseases are initiated by relatively small disturbances that are amplified through positive feedback loops (vicious cycles) and lead to the destabilization of organism physiology and, eventually, to death**. In the following sections, I will describe specific vicious cycles likely underlying five major ARDs: atherosclerosis, hypertension, diabetes, Alzheimer's and Parkinson's. Potential ways to interrupt these cycles will also be suggested. Finally, I will propose an evolutionary explanation for the existence of these vicious cycles.

The vicious cycle of atherosclerosis

Cardiovascular diseases are the leading cause of death worldwide. Atherosclerosis and hypertension are the major factors responsible for the myocardial infarction (heart attack) and the cerebrovascular insult (stroke). These events typically occur upon the rupture of a vulnerable atherosclerotic plaque in the artery wall, leading to thrombosis of a coronary artery or an artery in the brain. Vulnerable plaques are defined primarily by the large lipid-rich necrotic core and thin fibrous cap[6]. They progressively develop from initial benign fatty streaks, which are present already in infancy[7-9]. Notably, the number of advanced lesions and the lesion size grow exponentially with age[7,9], whereas the fibrous cap thins[10,11].

I propose the following mechanism involved in the development of a necrotic lipid core (for a similar idea, see Ref[12]):

(1) Low-density lipoprotein (LDL) concentrations in the bloodstream and in the subendothelial space (intima) of arteries are in dynamic equilibrium[13] and do not substantially change with age[14]

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(2) Endothelial cells and vascular smooth muscle cells produce reactive oxygen species (ROS) via NADPH oxidases (NOX), which are required for normal intercellular signaling[15,16]

(3) Occasionally, some LDL in the intima becomes oxidized by ROS from NOX, forming oxLDL[17]

(4) oxLDL promotes the expression of adhesion molecules on endothelial cells that mediate recruitment of monocytes from the bloodstream[8,18-22]

(5) Recruited monocytes differentiate into macrophages that engulf oxLDL (mainly via the scavenger receptor CD36) and try to digest it[23-26]

(6) Phagocytosis of macrophages is accompanied by the release of large amounts of ROS via NOX-2 (the so called respiratory burst)[27,28]

(7) These ROS oxidize more LDL[12,29]

Steps 4 to 7 are repeated many times: the key vicious cycle is formed (Figure 2A). It is supplemented by additional cytokine-mediated positive feedback loops: oxLDL-activated macrophages release cytokines that enhance macrophage recruitment, oxLDL phagocytosis, ROS production and cytokine production, either directly or via the activation of T cells, endothelial cells and vascular smooth muscle cells[30,31]. Eventually, the amount of oxLDL generated by the congregation of ROS-producing macrophages exceeds their digestion capacity[32]. Macrophages turn into foam cells and die, leading to necrotic core growth[33]. Fibrous cap thinning is likely the byproduct of the vicious cycle of lipid core growth. Indeed, thinning occurs due to the destruction of extracellular matrix collagens and elastins by matrix metalloproteinases (MMPs)[34], which are secreted by macrophages that are recruited to the lipid core[35] (Figure 2A).

In the end, it is not very important what exactly initiates this feedback loop – an increase in the intimal concentration of LDL, e.g. due to excessive fatty meal intake, or the burst of ROS production by endothelial cells, e.g. due to angiotensin II stimulation, or both factors. After all, fatty streaks are present already in the human fetus[8]. Once the loop is formed, it becomes self-sustainable, so no repeated external influences are required to keep it functioning. The only possible way to prevent the progression of the disease is to interrupt the vicious cycle, at any of its steps.

For example, ROS can be scavenged with antioxidants, decreasing oxLDL formation; the expression of adhesion molecules on endothelial cells can be downregulated, interfering with monocyte recruitment; CD36 and NOX-2 expression or function in macrophages can be inhibited, abolishing oxLDL uptake and ROS production; the secretion of cytokines and MMPs by macrophages can also be reduced to prevent additional feedback loops and fibrous cap degradation. However, ROS, adhesion molecules, macrophages, CD36, NOX-2, cytokines and MMPs also serve many physiological functions in the arterial wall and elsewhere, so their complete elimination or inhibition will do more harm than good. This may explain the failure of traditional approaches based on antioxidants and inhibitors. Instead, the success can be achieved when these same targets will be hit selectively in the atherosclerotic plaque. This approach will likely be based on the delivery of inhibitor cocktails within nanoparticles guided by plaque-specific markers[36-38].

The vicious cycles of hypertension

As mentioned in the previous section, hypertension (high blood pressure) is one of the two leading causes of acute cardiovascular events, along with atherosclerosis. For example, the rupture of a vulnerable plaque or of an aneurism is more likely to occur when blood pressure is high. Also, hypertension imposes greater load on the heart muscle, which may lead to the congestive heart failure.

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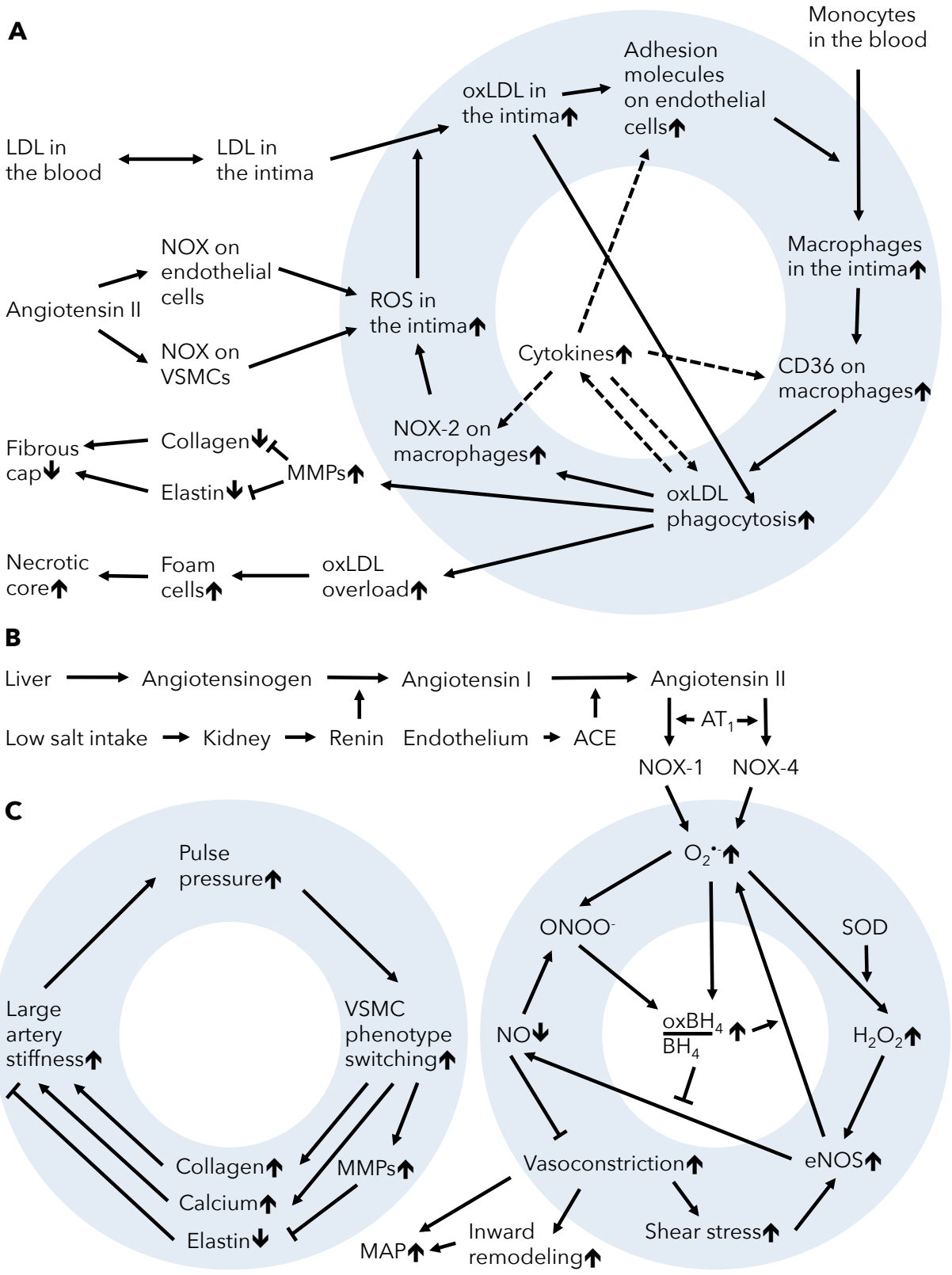

Figure 2. The vicious cycles of atherosclerosis (A), increased mean arterial pressure (B) and increased pulse pressure (C). Short vertical arrows indicate increase (↑) or decrease (↓) in parameters that are likely to change exponentially or logistically with age. LDL – low density lipoprotein, oxLDL – oxidized LDL, MMPs - matrix metalloproteinases, NOX – NADPH oxidase, ROS – reactive oxygen species, VSMCs – vascular smooth muscle cells, ACE – angiotensin converting enzyme, AT_1 – angiotensin II receptor type 1, $O_2^{\bullet-}$ - superoxide, SOD – superoxide dismutase, eNOS – endothelial nitric oxide synthase, NO – nitric oxide, $ONOO^-$ - peroxynitrite, BH_4 – tetrahydrobiopterin, $oxBH_4$ – oxidized BH_4, MAP – mean arterial pressure.

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Hypertension can be subdivided into two types: increased mean arterial pressure and increased pulse pressure. Mean arterial pressure increases until age 60, when it reaches a plateau, whereas pulse pressure increases exponentially until the oldest observed age[39]. Thus, these two types of hypertension likely correspond to the two subtypes of hypertension mortality discussed in the introduction. Indeed, the contribution of the minor mortality component to overall mortality from hypertension starts to decrease at age 60 (Figure 1).

An increase in mean arterial pressure occurs upon an increase in cardiac output and/or total peripheral (systemic) vascular resistance[40,41]. The former is often responsible for prehypertension, whereas the latter mediates the established form of the disease[42-44]. Systemic vascular resistance is determined predominantly by the lumen diameter of small arteries and arterioles, also called resistance vessels[40]. Increased vascular resistance can thus be due to increased vasoconstriction or impaired vasodilation.

The constriction of blood vessels can be caused by angiotensin II released by the renin-angiotensin-aldosterone system, mostly in response to decreased salt reabsorption in the kidneys and the related loss of blood volume[45,46]. However, the activity of the renin-angiotensin-aldosterone system does not increase with age, but rather decreases, both in hypertensives and in controls[42,47-49]. The hyperactivity of the sympathetic nervous system can also result in marked vasoconstriction[50,51]. Interestingly, the sympathetic activity correlates positively with mean arterial pressure[52,53] and age[54]. However, the complexity of the central nervous system and our insufficient knowledge of it hinder the identification of potential vicious cycles. The relaxation of blood vessels is achieved chiefly by nitric oxide (NO), which is produced by endothelial nitric oxide synthase (eNOS) primarily in response to shear stress[55]. Reduced NO availability, endothelial dysfunction and impaired vasodilation are commonly implicated in essential hypertension[56-58].

I propose the following chain of events underlying the reduced NO availability (a similar concept has been suggested before[59]):

(1) Low salt intake activates the renin-angiotensin-aldosterone system, which releases angiotensin II[60,61]

(2) Angiotensin II activates its receptor (AT_1) on endothelial and vascular smooth muscle cells[62]

(3) AT_1 triggering leads to the increased expression and activation of NOX-1 and NOX-4 that start to produce superoxide ($O_2^{\bullet-}$)[63-66]

(4) Some $O_2^{\bullet-}$ dismutates to hydrogen peroxide (H_2O_2), either spontaneously or with the help of superoxide dismutase (SOD)[67]

(5) H_2O_2 promotes the activation of eNOS, which increases the synthesis of NO[68]

(6) Some $O_2^{\bullet-}$ reacts with NO, producing peroxynitrite ($ONOO^-$)[69]

(7) Both $O_2^{\bullet-}$[70] and $ONOO^-$[71] oxidize and inactivate eNOS cofactor tetrahydrobiopterin (BH_4)

(8) When the ratio of intact BH_4 to oxidized BH_4 derivatives decreases, eNOS undergoes uncoupling and switches from the production of NO to the production of $O_2^{\bullet-}$[59,72,73]

Steps 4 to 8 comprise the vicious cycle (Figure 2B). After some time, BH_4 becomes severely oxidized, most eNOS enzymes switch to producing $O_2^{\bullet-}$, NO synthesis dramatically reduces, and NO availability approaches zero. These events impair the relaxation of small arteries and arterioles, increase systemic vascular resistance, and ultimately raise mean arterial pressure to the observed plateau. Moreover, prolonged functional vasoconstriction causes inward eutrophic remodeling of small arteries, leading to the structural fixation of the abnormality[74]. Importantly, inward eutrophic remodeling can be induced by the chronic inhibition of NO production[75]. Fortunately, vasodilator agents

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have been shown to reverse this process by inducing outward remodeling[76]. Interestingly, the same vicious cycle may be present in large arteries where it may be interwoven with the atherosclerosis cycle, as both cycles generate, and are propelled by, increasing ROS levels.

Most effective strategies to interrupt this vicious cycle would be using the scavengers of $O_2^{\bullet-}$[77] and $ONOO^-$[78] and increasing SOD expression and BH_4 synthesis[79] in endothelial and vascular smooth muscle cells, whereas exogenous supplementation of BH_4 is ineffective due to its rapid oxidation and slow reduction[80,81]. Inhibiting vascular NADPH oxidases, AT_1 or angiotensin II synthesis also appears much less effective, as once the positive feedback loop (steps 4-8) is initiated, these molecules are no longer critical for the progression of the disease.

Pulse pressure is determined predominantly by the elasticity (or stiffness) of large arteries[40,41]. Arterial stiffening causes increased pulse pressure by attenuating the compensatory arterial stretching during systole and shrinking during diastole[82]. Arterial stiffening progresses exponentially[83] and appears to predict hypertension[84-89] and acute cardiovascular events[90]. Interestingly, pulse pressure[91] and systolic pressure[83] also predict arterial stiffening, suggesting the possibility of a positive feedback loop between hypertension and arterial stiffening[39,92-94]. Arterial stiffening results from the fragmentation of elastin, deposition of collagen and calcium, as well as elastin and collagen crosslinking[95,96]. It is not known how exactly hypertension triggers these processes, but the transformation of vascular smooth muscle cells in the arterial wall to the synthetic and osteoblastic phenotypes in response to mechanical forces is the likely candidate[97,98] (Figure 2C). Thus, further research into the vascular smooth muscle cell phenotype switching will help to uncover the ways to interfere with or even reverse this pathological remodeling of large arteries.

The vicious cycles of diabetes

Diabetes mellitus is the group of metabolic diseases characterized by increased blood glucose levels (hyperglycemia). There are two major types of diabetes. Type 1 (insulin-dependent) diabetes is caused mainly by the autoimmune destruction of insulin-producing beta cells in the islets of Langerhans in the pancreas and accounts for 5-10% of all cases[99-101]. Type 2 (non-insulin-dependent) diabetes also involves the dysfunction and death of beta cells, but is triggered primarily by insulin resistance and comprises 90% of cases[102,103]. Diabetes may lead to serious health complications, such as neuropathy, retinopathy, nephropathy, seizures, nonketotic hyperosmolar coma, blindness, muscle wasting, chronic kidney disease, ketoacidosis, foot ulcers, cardiovascular diseases, and finally death.

The events underlying the development of type 1 diabetes are typical for a T cell-mediated autoimmune disease[104] and can be described as follows:

(1) The infection of beta cells by a virus leads to their destruction and the subsequent capture of their self-antigens by antigen-presenting cells, which include macrophages and dendritic cells[105,106]; alternatively, viral or bacterial antigens that mimic beta-cell antigens are captured by antigen-presenting cells elsewhere in the body[107-109]

(2) Antigen-presenting cells migrate to lymph nodes and present captured antigens on Major histocompatibility complex class II (MHC II) in the form of short peptides[110]

(3) Naïve $CD4^+$ T cells with T-cell receptor (TCR) complementary to the presented peptide-MHC II complex become activated, license antigen-presenting cells, proliferate, exit the lymph node and migrate to the pancreas[110,111]

(4) Licensed antigen-presenting cells activate naïve autoreactive $CD8^+$ T cells by antigens cross-presented on MHC I, inducing

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their proliferation and migration to the pancreas[111-113]

(5) In the pancreas, $CD4^+$ T cells are restimulated by resident antigen-presenting cells via peptide-MHC II and start to secrete cytokines, which stimulate $CD4^+$ T cells, $CD8^+$ T cells and antigen-presenting cells[114,115]

(6) Activated $CD8^+$ T cells recognize self-antigens in complex with MHC I on beta cells and destroy these cells with FasL, perforins and granzymes[116,117]

(7) Antigen-presenting cells capture beta-cell debris that contain novel antigens and epitopes[118,119]

Steps 2 to 7 form the vicious cycle, leading to epitope spreading[120,121] and the progressive destruction of beta cells (Figure 3A). The avidity maturation of T-cell clones may also be involved in the escalating kinetics of the disease[122]. The loss of beta cells leads to acceleratingly decreasing glucose sensitivity, resulting in exponentially increasing blood glucose and the onset of diabetes[123,124]. Treatment for type 1 diabetes clearly should be directed against the escalation of autoimmune response in the pancreas. The selective elimination[125,126] or suppression[127] of cytotoxic and helper T cells bearing receptors against beta-cell antigens appears to be the most promising approach.

To explain the progressive development of type 2 diabetes, I propose the following sequence of events (see Ref[128] for a similar concept):

(1) Overeating (the consumption of more calories than spent) leads to obesity

(2) Obesity causes insulin resistance, likely through the dysfunction[129,130] and/or inflammation[131-136] of the adipose tissue. However, insulin resistance does not directly participate in the main vicious cycle of diabetes, because it appears early, when blood glucose levels are still normal, and then changes little, when glucose concentrations in the blood rise exponentially, and beta-cell function dramatically deteriorates[137-141]

(3) Nevertheless, insulin resistance places increased demand on pancreatic beta cells, as they need to produce more insulin to keep blood glucose levels within the normal range[142-144]

(4) An increase in the secretion of insulin per beta cell leads to an increase in the endoplasmic reticulum stress[145] and apoptosis[146-150] of beta cells

(5) A decrease in the number of functional beta cells leads to a decrease in the total pancreatic rate of insulin secretion relative to a given glucose level (decreased glucose sensitivity, $\Delta I/\Delta G$, HOMA-B)[138,140,151], assuming that the glucose sensitivity of each individual beta cell does not change

(6) A decrease in the total relative insulin secretion rate leads to a decrease in the insulin-mediated glucose uptake by liver, muscle and adipose tissues and a decrease in the insulin-mediated suppression of hepatic glucose production (both also relative to glucose levels in blood)[152-154], thus increasing blood glucose levels

(7) Moreover, a decrease in the ratio of beta to alpha cells[147,148,150] (due to beta-cell apoptosis) leads to a decrease in the suppression of glucagon secretion[155-157], promoting an increase in hepatic glucose production[158] and a further increase in glucose levels

(8) An increase in blood glucose levels causes each beta cell to secrete more insulin[159]

Steps 4 to 8 form the vicious cycle, leading to impaired glucose tolerance and type 2 diabetes (Figure 3B). It should be noted that the same vicious cycle can be operating in type 1 diabetes in parallel with the autoimmune destruction cycle, as the number of beta cells and glucose sensitivity decrease, and endoplasmic reticulum stress increases, in both cases[123,160,161]. In fact, both diseases might differ only by the factor (autoimmune response vs. insulin resistance) that triggers the vicious cycle.

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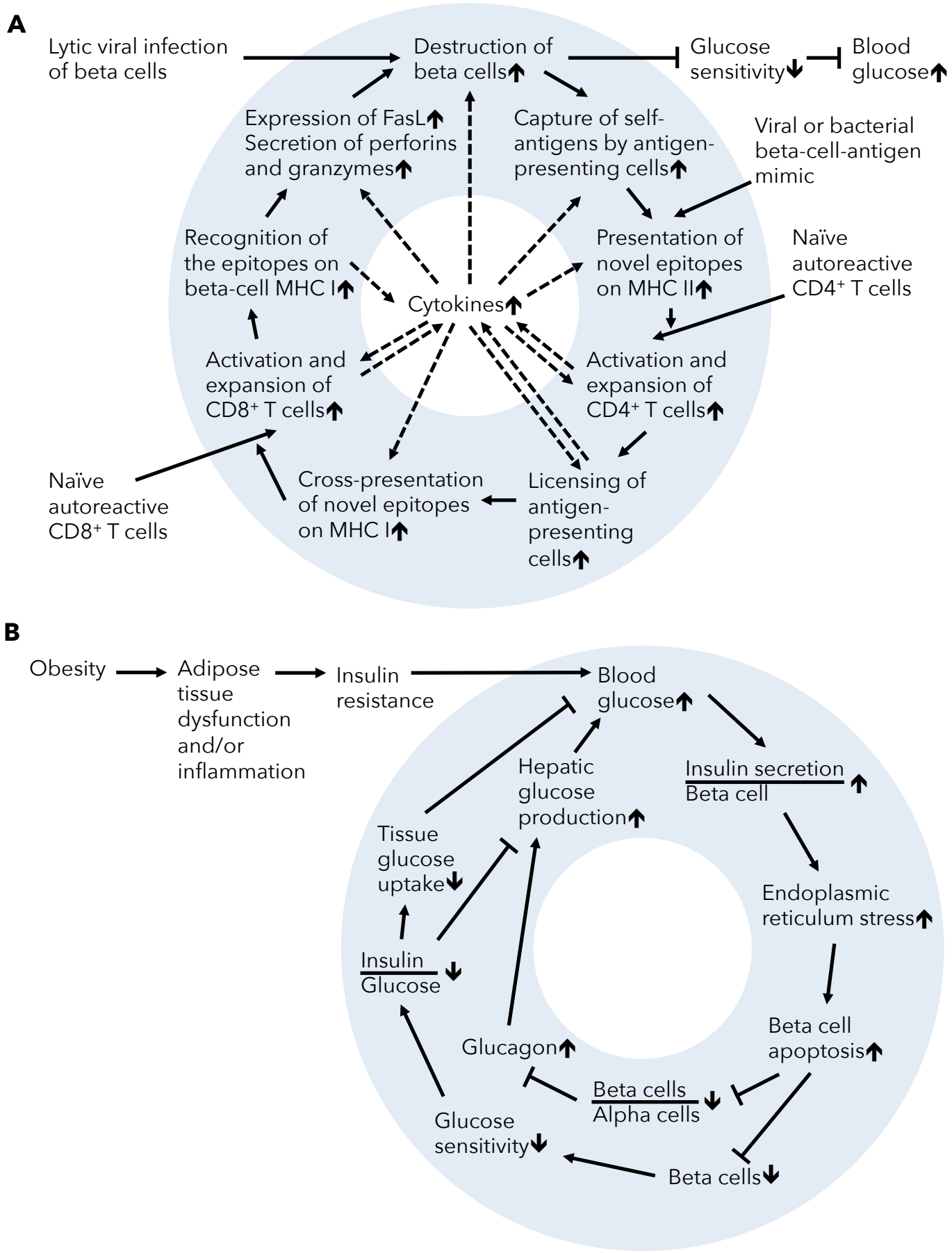

Figure 3. The vicious cycles of type 1 diabetes (A) and type 2 diabetes (B). Short vertical arrows indicate increase (↑) or decrease (↓) in parameters that are likely to change exponentially or logistically with age. MHC – major histocompatibility complex, FasL – Fas ligand.

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To break this cycle, blood glucose needs to be lowered for the period of time sufficient for the pancreas to recover proper beta cell numbers. This can be achieved by the supplementation of exogenous insulin, by decreasing insulin resistance, by inhibiting glucagon secretion and hepatic glucose production, or by diminishing glucose reabsorption in kidneys. Increasing insulin sensitivity will also help to prevent the re-initiation of the cycle.

The vicious cycle of Alzheimer's disease

Alzheimer's disease is the most common neurodegenerative disorder in the elderly. The progressive deterioration of memory, reasoning and language skills leads to confusion, anxiety, frustration and abnormal behavior. Finally, death from aspiration pneumonia may occur. Alzheimer's disease is classically characterized by the build up of amyloid plaques in the brain, along with the appearance of neurofibrillary tangles, synaptic degeneration and neuronal death[162,163]. Amyloid plaques consist of amyloid fibrils, which in turn are composed of amyloid beta (Aβ)[164,165]. Aβ is a peptide of unknown function, cleaved from amyloid precursor protein (APP) by the sequential action of β- and γ-secretases[166].

The elongated form of Aβ (Aβ42) can be released as the result of incomplete processing, which is dramatically increased by mutations in APP or presenilins, the key subunits of γ-secretase[167-169]. If these mutations are inherited, the familial autosomal dominant early-onset Alzheimer's disease appears, comprising around 5% of total Alzheimer's cases, with the rest 95% called late-onset (sporadic) Alzheimer's. Moreover, all known mutations in early-onset familial Alzheimer's map to APP or presenilins, and almost all of them lead to the increased production of Aβ42[167-169].

Aβ42 has a particular conformation that is prone to oligomerization and subsequent fibril formation[170-173]. The few mutations that do not lead to increased Aβ42 formation nevertheless appear to promote Aβ oligomerization by other means[169,174,175]. Recent studies suggest that Aβ oligomers/protofibrils, rather than mature fibrils, are the primary neurotoxic species[171,176-179]. The most remarkable feature of amyloid is that its molecules in an altered conformation can induce a similarly altered conformation in normal amyloid molecules, as in prion diseases[180-184]. This amyloid property allows the chain reactions of amyloid oligomerization to occur.

I propose the following sequence of events that leads to the formation of toxic Aβ oligomers in sporadic Alzheimer's disease:

(1) In the area of adult neurogenesis, such as the hippocampus[185-193], a mutation in APP or presenilin spontaneously arises in one cell, leading to the increased production of Aβ42[167,168] (mutations are unlikely to occur in postmitotic cells, such as differentiated neurons, as they normally emerge during DNA replication)

(2) Aβ42 molecules have an altered conformation that induces their oligomerization (primary nucleation/seed formation) when their local concentration is high enough, such as that created by the somatic mutation of APP or presenilin[170-173,194,195]

(3) Aβ42 monomers, which are released at low rates by cells without mutations in APP and presenilin, as well as Aβ40 monomers released at high rates, attach to the ends of growing Aβ protofibril chains[171,172,196]

(4) Crucially, Aβ protofibrils also serve as secondary nucleation sites, catalyzing the formation of new oligomers, both from Aβ42 and Aβ40[173,194,195]

Steps 3 and 4 are continuously repeated, constituting the chain reaction and resulting in the progressive spread of Aβ pathology[195] (Figure 4A). Recent studies also show that Aβ oligomers can be taken up by cells and can

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spread from synapse to synapse along the defined neurological pathways, such as from the hippocampus to cortical areas[182,183]. Aβ oligomers can exert their toxicity directly[171,176-179,197-199] or via other molecules, such as tau[200-202], the major component of neurofibrillary tangles, recently also shown to exist in the oligomeric form[203]. Aβ aggregates can also induce the activation of microglia via the scavenger receptor CD36[204,205]. Importantly, the deposition of Aβ with age has been shown to follow sigmoidal curve[206], in line with the *in vitro* kinetic models of Aβ aggregation[171,173,194,195] and the logistic growth of Alzheimer's disease mortality (Figure 1).

Considering the hypothetical mechanism proposed here, the only effective way to prevent Alzheimer's disease progression would be to interfere with the chain reaction of Aβ oligomerization. Chemical substances that bind to the oligomer/protofibril ends and block the addition of intact Aβ molecules[207-209], as well as substances that interfere with secondary nucleation[210], appear to be the best candidates.

The vicious cycles of Parkinson's disease

Parkinson's disease is the second most common neurodegenerative disorder of old age. Its major symptom is the progressive loss of motor control, manifesting in resting tremor, muscle rigidity, and bradykinesia (slowness), as well as balance, posture and walking problems. Cognitive deficiencies may develop at the late stages of the disease, resulting in 'Parkinson's disease dementia'. If they develop earlier than motor deficits, the disorder is called 'dementia with Lewy bodies'.

The pathology of Parkinson's disease is characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, which project to the striatum[211]. It is widely agreed that the loss of dopaminergic stimulation of the striatum is the direct cause of motor control deficits[212]. The remaining neurons in the substantia nigra show characteristic inclusions called Lewy bodies and Lewy neurites, which are composed primarily of alpha-synuclein (αS) and ubiquitin[213-218]. When cognitive deficiencies are present alongside impairments in motor control, Lewy bodies and neurites are found in many other brain areas besides the substantia nigra[219].

Mutations in αS are the only known mutations necessary and sufficient for the development of familial autosomal dominant early-onset Parkinson's disease[220-222], implying the gain of toxic function by αS. Interestingly, the duplications and triplications of αS gene cause the same disease as missense mutations in αS, with the rate of progression proportional to the gene dosage[223-226], indicating the toxicity of wild-type αS in elevated concentrations. Additionally, mutations in three other proteins – parkin[227], PINK-1[228] and DJ-1[229] – are known to cause autosomal recessive early-onset Parkinson's, suggesting the loss of protective function by these proteins. Altogether, early-onset forms constitute only 5-10% of all Parkinson's cases but likely highlight the key players in the sporadic disease. Indeed, polymorphisms in the αS gene and promoter are the most significant SNPs associated with sporadic Parkinson's disease[230-237].

The mechanism of Parkinson's disease initiation and progression might be analogous to that of Alzheimer's disease:

(1) A heterozygous mutation in αS[220-222] or the multiplication of the αS gene[223-225] arises in a neural progenitor in the subventricular zone[238], which then travels to the striatum[239] (as explained above, mutations are very unlikely to occur in postmitotic cells, such as differentiated neurons, as they normally emerge during DNA replication; the subventricular zone and the striatum are directly connected to the substantia nigra via dopaminergic afferents[240-242]; finally, the spontaneous somatic mutation explains the

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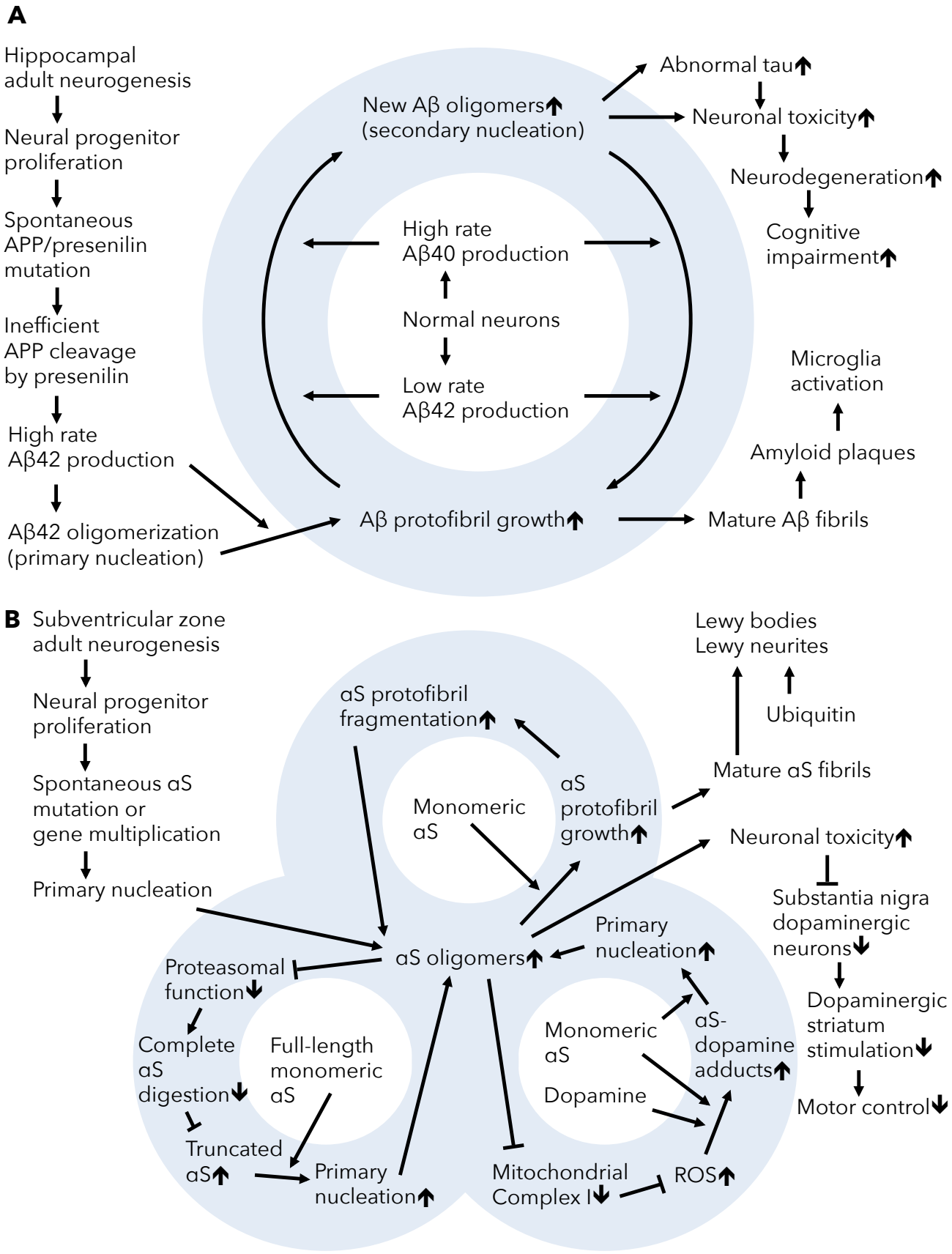

Figure 4. The vicious cycles of Alzheimer's disease (A) and Parkinson's disease (B). Short vertical arrows indicate increase (↑) or decrease (↓) in parameters that are likely to change exponentially or logistically with age. APP – amyloid precursor protein, Aβ – amyloid beta, Aβ42 – 42 amino acids long Aβ, Aβ40 – 40 amino acids long Aβ, αS – alpha synuclein, ROS – reactive oxygen species.

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asymmetric disease manifestation and the random "selection" between the right and the left hemisphere[243,244], underscored by the symmetric manifestation when the mutation is inherited and hence present in all cells[245,246])

(2) A mutation in αS[247-252], or the locally increased concentration of αS[253] due to the multiplication of the αS gene[254,255], promotes αS oligomerization (primary nucleation/seed formation)

(3) Oligomers grow into protofibrils by the attachment of monomeric wild type αS[252]

(4) Growing protofibrils may fragment to oligomers (secondary nucleation/seed formation)[252]

Steps 3 and 4 are continuously repeated, constituting the chain reaction and resulting in the progressive spread of αS pathology[182,183] (Figure 4B). Similarly to Alzheimer's disease, substances that bind to αS oligomers/protofibrils and block the addition of monomers appear to be the perfect drug candidates for the treatment of Parkinson's disease[256].

An additional positive feedback loop may exist[257] (Figure 4B):

(1) As oligomers spread, they interfere with proteasomal functions[258,259], leading to the formation of incompletely digested (truncated) αS[257,260]

(2) Truncated αS is aggregation-prone[261,262] and promotes the aggregation of full-length monomeric αS into oligomers and fibrils[257,260,263,264]

Elucidating the exact mechanism of proteasome malfunction and αS truncation may help to design drugs that halt this vicious cycle. However, more promising approaches appear to be the inhibition of the attachment of full-length αS to the truncated one or the inhibition of protofibril elongation[265].

Accumulating oligomers are toxic to neurons[266-268]. On the contrary, mature fibrils in Lewy bodies and neurites may be neuroprotective by sequestering toxic oligomers[217]. However, the elegant justification of Lewy body-associated toxicity has been proposed based on the stable percentage of Lewy body-bearing neurons[269]. Indeed, Lewy bodies can represent the failure of the ubiquitin-proteasome system[217,259,270], e.g. due to αS "poisoning" [258,259].

The third vicious cycle involving mitochondria may be present (Figure 4B):

(1) Spreading αS oligomers inhibit mitochondrial Complex I[271,272]

(2) The inhibition of Complex I leads to an increase in ROS[273]

(3) ROS modify αS and dopamine, promoting αS-dopamine covalent binding[274-277]

(4) Dopamine adducts promote the conversion of αS into toxic oligomers/protofibrils but prevent the formation of mature fibrils[274-277]

This mitochondrial cycle is supported by the involvement of parkin[278], PINK-1[279] and DJ-1[280-282] in mitochondrial maintenance and antioxidant activity, by deficient Complex I activity in Parkinson's disease patients[271,283,284], as well as by the induction of parkinsonism by mitochondrial Complex I inhibitors and ROS inducers[285-289]. Crucially, toxin-induced parkinsonism is progressive[290,291] and mediated by αS[292]. In fact, intriguing evidence indicates that an exposure to mitochondrial toxins via the gastrointestinal tract can lead to the accumulation of αS in the enteric nervous system and spreading of the pathology along the nerve fibers to the substantia nigra[293,294]. Mitochondria-targeted antioxidants[295] and substances that prevent the binding of oligomers to Complex I may help to slow down this vicious cycle. Surprisingly, the clinical trial of MitoQ showed no benefit for Parkinson's disease progression[296], which may indicate that the mitochondrial cycle is not the major one in the sporadic patients.

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Evolutionary perspective

Why do diseases exist? Why has the perfect body design not evolved in 3 billion years? The answer is that the perfect design cannot exist even in theory. A species design is the result of the preceding evolutionary adaptation to the environment, so the perfect design would mean the perfect adaptation. But the environment is continuously changing, so the design of all species that inhabit it must change as well, to remain adaptive. As there is no possibility for a species to "predict" how the environment will change, the processes of genetic shuffling (sexual reproduction, meiosis, homologous recombination) emerged to constantly vary the design, "in hope" that at least some of the variants will be adapted sufficiently well to the new environment, and thus survive and procreate. In less anthropomorphic terms, species that acquired genetic shuffling adapted faster to the ever-changing environment and outcompeted other species.

A design that is created by random assembling from pieces (of genetic information) is bound to be imperfect. In case of severe misfortune, a design fails during natal development. More fit individuals may die in childhood or puberty. Those who have managed to live to a ripe-old age may be considered almost perfect (for the time). Similar logic explains interspecies variability in the lifespan. A species whose genetic pool allows better adaptation of the individuals to the current environmental niche that they occupy will have a longer average individual lifespan, relative to a species not fitting its current environment so well (but which may nevertheless survive by boosting the production of new individuals).

Thus, there is no surprise that something in our bodies goes wrong as we grow and age. The question is why do some systems of the body fail more often than others? We can postulate that **most failures occur in those systems that deal with the most rapidly changing environmental factors, as the evolution of the species struggles to keep up with them.** One group of such factors appears to be the rapidly evolving viruses, bacteria and parasites[297,298]. Hence, many failures are expected in the immune system, resulting both in the inability to counteract pathogens (lethal infections) and in the attack on the self (autoimmune disorders). Indeed, the immune system is central to the development of atherosclerosis, type 1 diabetes and insulin resistance (Figure 5, see also the inflammaging theory[299]).

Another rapidly evolving factor is the human civilization itself. Thus, many diseases are expected to be due to modern lifestyles and technogenic environments – overeating, the lack of physical activity, the lack of sleep, close work (books, computers, smartphones), smoking, alcohol consumption, polluted air, processed foods, artificial lighting, noise pollution, electromagnetic pollution, information overload, etc. Insulin resistance is thought to be caused by obesity, which results from overeating, the lack of physical activity and possibly the consumption of processed foods (Figure 5, see also Ref[300]).

Some factors do not change with time but are nevertheless impossible to be fully adapted to. Somatic mutations in DNA are one such factor, and lead to cancer[1]. Alzheimer's and Parkinson's diseases also seem to be initiated by spontaneous mutations in Aβ and αS (Figure 5, see also the DNA damage theory of aging[301]). ROS are another example of the factor difficult to adapt to. Although aerobic organisms have developed protective systems against these harmful but inevitable byproducts of respiration, and even harnessed them for various antimicrobial and signaling purposes, they are still playing with fire. This is illustrated by the crucial role of ROS in the pathogenesis of atherosclerosis and endothelial dysfunction (Figure 5, see also the free-radical theory of aging[302]).

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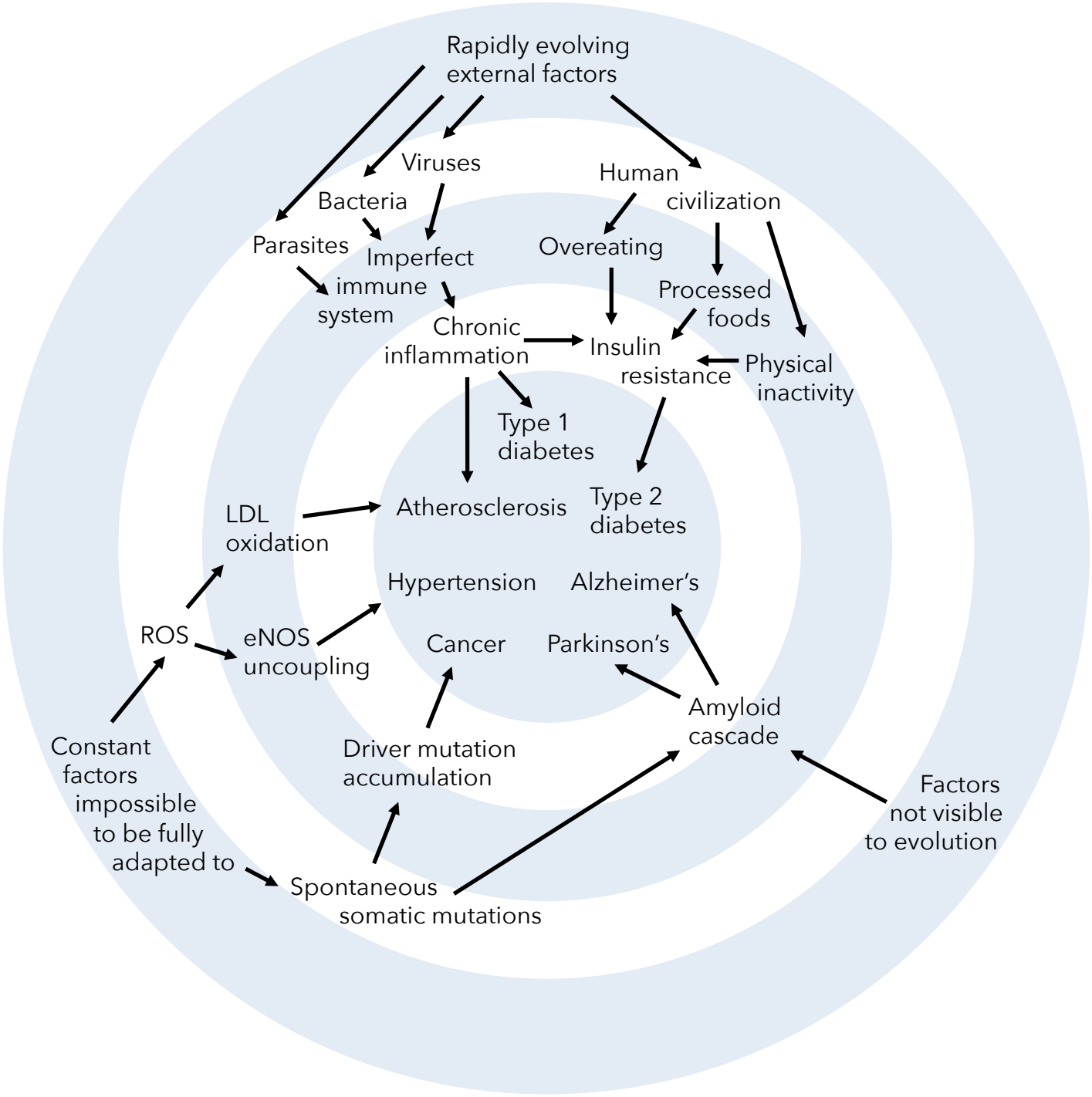

Figure 5. Factors that trigger age-related diseases. ROS – reactive oxygen species, LDL – low density lipoprotein, eNOS – endothelial nitric oxide synthase.

Other dangerous toys are amyloid proteins, such as Aβ and αS, that upon a single mutation, truncation or modification, or even simply in increased concentration, can form self-amplifying toxic oligomers. It would probably be possible for the evolution to make these proteins benign, lacking the ability to aggregate, grow and multiply.

Unfortunately, the side effects of amyloid accumulation were not visible to the evolution until the 20th century, when a dramatic increase in life expectancy has occurred[303]. For hunter-gatherers, the life expectancy at age 5 was additional 25 to 50 years[304], which means that an average human being who survived through infancy and early childhood died at age 30 to 55 for most of our species

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history. Interestingly, 95% of Alzheimer's disease patients have the sporadic late-onset form, which by definition appears after age 65[305]. The cutoff for late-onset Parkinson's disease (90-95% of patients) varies from age 40 to age 65 according to investigator preferences, and usually is set at age 50[306]. Apparently, there was no sufficient selective pressure on our species to develop countermeasures against these diseases (Figure 5).

The factors described above are difficult for evolving species to adapt to. In fact, **diseases may represent failed adaptive responses. Moreover, a successful response that is protective during the evolutionarily visible lifespan may turn out to be harmful at older ages.** In fact, atherosclerosis can be viewed as the sanitary response of macrophages to atypical substances in the arterial wall, which fails due to the ROS spillover. Arterial stiffening may represent the preventive response of vascular smooth muscle cells to the threat of arterial rupture due to increased stretching, which works but results in increased pulse pressure, leading to other cardiovascular events during the evolutionarily irrelevant part of lifespan. Type 1 diabetes is presumably the immune response to a viral infection that goes wrong because of molecular mimicry or simply due to the fact that immune response is a "delicate war" and any loss of balance may lead to dire consequences. Type 2 diabetes results from the attempt of beta cells to compensate for lifestyle-imposed insulin resistance by maximizing their insulin production, which works for some time but finally leads to the endoplasmic reticulum stress and massive beta-cell death. Insulin resistance itself could be the protective physiological response to obesity (see also the antagonistic pleiotropy theory of aging[307]).

The final question is why ARDs are based on positive feedback loops? Positive feedback loops may play the central role in diseases simply because they represent processes with the dynamics that is very difficult for the body to handle. Unlike processes with the linear dynamics, which have a predictable pace and thus are easy to counteract, processes involved in positive feedback loops have the exponential dynamics, which is deceptively slow for most of the timespan but then accelerates very rapidly and unexpectedly. Thus, **the body can efficiently neutralize damage arising at a constant rate but cannot cope with damage that appears at an ever-increasing rate.** This is likely because during the evolutionarily relevant 55 year lifespan[304] the body is exposed only to the slow part of the exponent (Figure 1), thus the evolution does not "see" the hazard and the "need" to develop the negative feedback loops to keep the system in check. In other words, the harmful effects of these vicious cycles were not visible to the evolution as they occur after the historical average age of death for our species, and thus no countermeasures have evolved.

Future avenues

Although much effort has been spent to identify the most likely vicious cycles underlying each ARD, it was necessarily based on the limited and often controversial studies available to date. It is thus quite possible and even expected that when our knowledge expands and deepens, the cycles proposed here would be modified, or even replaced by the newly discovered cycles. To aid those future efforts, I would like to suggest a simple rule. Factors triggering the cycle in question should be distinguished from factors directly participating in that cycle. The former will likely show an association with the disease in epidemiological studies but do not have to change with age. The latter will also show an epidemiological association but in addition must change with age, preferably exponentially or logistically. The examples of triggering factors are LDL and angiotensin II

concentrations in the blood, type 1 diabetes-associated viruses and obesity. The examples of cycle-participating factors are the amount of oxLDL in the plaques and Aβ oligomers in the brain, the level of $O_2^{\bullet-}$ in the endothelium and glucose in the blood, pulse pressure, and the number of activated T cells in the pancreas.

This study showed that vicious cycles underlying ARDs are quite diverse and unique, casting serious doubts on the possibility of discovering the single molecular cause of aging and developing the single anti-aging pill. Rather, each disease appears to require an individual approach. The theory of vicious cycles helps to identify the most promising targets for the treatment of each ARD. In the end, aging is relevant mostly as an umbrella term for ARDs, as nobody apparently dies of "healthy aging"[308].

Methods

Data acquisition

'Underlying Cause of Death, 1999-2015' data were downloaded via Centers for Disease Control and Prevention Wide-ranging OnLine Data for Epidemiologic Research (CDC WONDER) online database: http://wonder.cdc.gov/controller/datarequest/D76.

The Underlying Cause of Death data are produced by the Mortality Statistics Branch, Division of Vital Statistics, National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention (CDC), United States Department of Health and Human Services (US DHHS). Data are based on death certificates for U.S. residents. Each death certificate identifies a single underlying cause of death and demographic data. Mortality information is collected by state registries and provided to the National Vital Statistics System. The population estimates are U.S. Census Bureau estimates of the July 1 resident populations, based on the year 2000 and the year 2010 April 1 census counts. Crude Rates are expressed as the number of deaths reported each calendar year per 100,000 population. Rates and Populations are reported as "Not Applicable" for any subset of ages 85 and over, because population estimates are not available for those ages. The full dataset description is available here: http://wonder.cdc.gov/wonder/help/ucd.html#.

Results were grouped by Age Groups, Single-Year Ages were selected in demographics tab, and 2000 to 2015 years were selected in the next tab. 1999 was not included due to population estimates based on a separate 1990 census. All other parameters were left at default settings. Then the data were downloaded separately for each disease, upon its selection in the ICD-10 Codes tab: Insulin-dependent diabetes mellitus (E10), Non-insulin-dependent diabetes mellitus (E11), Parkinson's disease (G20), Alzheimer's disease (G30), Essential (primary) hypertension (I10) and Atherosclerosis (I70).

Data analysis

For analysis, the crude mortality rates were imported into GraphPad Prism 5. Data were analyzed with Nonlinear regression. User-defined equations were created for the exponential and logistic functions:

Y=A*exp(b*x)

Y=A/(1+exp(-b*(x-t)))

The sum of 2 logistic functions was modeled as follows:

Y1=A1/(1+exp(-b1*(x-t1)))

Y2=A2/(1+exp(-b2*(x-t2)))

Y=Y1+Y2

The parameter *A* was constrained to "Must be between zero and 100000.0" and the parameters *b* and *t* to "Must be greater than 0.0". "Initial values, to be fit" for the parameters *A* and *t* were set to 1.0 and for the parameter *b* to 0.5. All other settings were left by default, e.g. Least squares fit and No weighting.

The hazard functions of the gamma, logistic, normal and Weibull distributions were also tested, but provided inferior fits

compared to the logistic function or did not converge at all. The hazard functions of the Gumbel and Gompertz distributions are equivalent to the exponential function. The hazard function of the Weibull distribution is equivalent to the power function. Both functions have no upper limit. The sum of two exponential functions did not provide a dramatic improvement in fit over the single exponential function.

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