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General introduction

Early molecular changes in Alzheimer Disease - can we catch the disease in its presymptomatic phase?

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Abstract

Alzheimer Disease (AD) is the most common form of dementia and characterized by deposition of amyloid-β (Aβ) plaques, neurofibrillary tangles consisting of hyperphosphorylated tau, atrophy and progressive neurodegeneration. While the familial, early onset form of AD is known to be caused by specific mutations in genes encoding presenilin 1, presenilin 2 or Aβ protein precursor, the underlying mechanisms leading to the development of sporadic AD are still not known. The major risk factors are, however, aging and APO-ε4. Here we review the latest evidence for the involvement of malfunctioning insulin signaling, dysfunction of mitochondria-associated membranes, cerebrovascular changes, increased oxidative stress and free radical formation, DNA damage, disturbed energy metabolism and synaptic dysfunction in early stages of AD. We focus on whether the changes in these processes precede or succeed the earliest symptoms in AD patients, i.e. minimal cognitive impairment. Since changes in Aβ processing are probably a key event in AD we also highlight the relationship of the above mentioned processes with the formation, secretion, aggregation and toxicity of Aβ. Based on our literature findings we propose a model in which insulin dysfunction, pathological cerebrovascular changes, dysfunction of mitochondria-associated membranes and/or synaptic changes are likely to interact with each other, thereby initiating and facilitating the development of AD pathology by accelerating the production and deposition of Aβ. Increased oxidative stress and free radical formation, DNA damage, disturbed energy metabolism and synaptic loss follow these events, but still occur very early in AD.
Introduction

Alzheimer Disease (AD) is the most common form of dementia among the elderly, contributing to up to 70% of all cases (WHO, 2012). In 2010, 36 million people suffered from AD, while this number increases by 7.7 million patients per year (Batsch and Mittelman, 2012). AD is characterized by progressive and selective loss of neurons and synapses, extracellular deposition of amyloid-ß (Aß) plaques and formation of intracellular neurofibrillary tangles (NFTs), dystrophic neurites and neuropil threads (neurofibrillary changes) composed of hyperphosphorylated tau protein, predominantly in brain regions involved in cognitive processes such as temporal, parietal and frontal cortex, hippocampus and amygdala (DeKosky et al., 2002; Ferretti and Cuello, 2011; Schon and Area-Gomez, 2010). Other hallmarks of AD are reduced cholinergic activity (DeKosky et al., 2002; Dubelaar et al., 2006), neuroinflammation (Akiyama et al., 2000; Cartier et al., 2005; Eikelenboom et al., 2011; Ferretti and Cuello, 2011) and atrophy (Swaab and Bao, 2011). 5-10% of AD cases are of early onset, starting at 50-60 years of age. Familial, early onset AD is caused by several mutations in the genes encoding presenilin 1 (PSEN1), presenilin 2 (PSEN2), or Aß protein precursor (AßPP), all of which lead to excessive production and deposition of Aß plaques and the characteristic AD pathology mentioned above (Wu et al., 2012). In this review, we focus on the sporadic form of the disease: in more than 90% of all cases, AD has a sporadic and late onset above the age of 65 years (Hellström-Lindahl et al., 2009). Age and the ε4 allele of apolipoprotein E (APOE-ε4) are generally accepted risk factors for the development of sporadic AD (Corder et al., 1993; Tol et al., 1999). In addition to APOE other (potential) susceptibility genes (CLU, PICALM, CR1) have been identified (Harold et al., 2009; Lambert et al., 2009). However, sporadic AD does not show any obvious inheritance patterns and causal mutations have not been identified (Bertram and Tanzi, 2004; Kamboh, 2004; Serretti et al., 2005).

Currently, a definitive diagnosis of AD can only be made based upon post-mortem analysis of the brain. The classification of the disease progression in post-mortem brain tissue of AD patients is based on six Braak stages (I-VI) according to the presence of neurofibrillary changes in distinct brain areas (Braak and Braak, 1991; see also Figure 1). In addition, Braak stage 0 is used to indicate the absence of any neurofibrillary changes. In Braak stage I and II no cognitive decline is appreciable, although slight amounts of hyperphosphorylated tau are present in the transentorhinal cortex. Braak stages I and II could represent what is often referred to as preclinical AD; the pathophysiological process, yet asymptomatic, that begins several years before the first symptoms of AD occur (Morris, 2005; Price et al., 2009). We should, however, acknowledge that others see AD-like neuropathology as a normal process of brain aging, which does not necessarily lead to
AD and can, therefore, not be seen as preclinical AD (Schmitt et al., 2000). In Braak stage III and IV the transentorhinal cortical area, the hippocampus and the limbic area show increased neurofibrillary changes and neurofibrillary pathology also starts to develop in the prefrontal cortex. Patients in Braak stage III-IV suffer from mild cognitive impairment (MCI). When memory problems are the most prominent cognitive deficit at this stage, researchers usually refer to amnestic MCI, a common prodromal state of AD (Gauthier et al., 2006). In Braak stage V-VI most parts of the neocortex are severely affected by neurofibrillary changes and patients are diagnosed with dementia (Braak et al., 2006). Aβ plaque deposition starts in the neocortex and spreads in later phases to the allocortex, the diencephalic nuclei and the striatum. In the latest phases Aβ plaques are found in the brainstem and the

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**Figure 1** Dysfunctions in biological processes precede cognitive impairment.

The occurrence of early dysfunctions in Alzheimer Disease (malfunctioning insulin signaling, dysfunctional mitochondria-associated membranes, cerebrovascular changes, oxidative stress, DNA damage, impaired energy metabolism, synapse dysfunction) is depicted in relation to the time course of clinical and pathological features (see text for details). Biological processes that could act potentially as disease-initiating factors have to become dysfunctional before cognitive decline appears. The neuropathological features neurofibrillary tangles and synapse loss gradually increase during disease progression and correlate with clinical symptoms of memory loss. Senile plaques appear before tangles and do not correlate with memory loss.
cerebellum (Braak and Braak, 1991; Thal et al., 2002). Importantly, Aβ plaque formation does not correlate with cognitive impairment in AD patients (Arriagada et al., 1992; Giannakopoulos et al., 2003; Shankar and Walsh, 2009; see also Figure 1).

As Aβ plaque deposition and neurofibrillary changes are the classical markers of AD, they have been studied intensively as disease-initiating factors. Imaging of Aβ plaques with Pittsburgh compound B (PiB) has shown that the amount of Aβ plaques can predict the progression from cognitive normality to AD (Morris et al., 2009). This is supported by many other studies showing that the presence of Aβ plaques in cognitively intact individuals is not benign, but can lead eventually to dementia (reviewed in Vlassenko et al., 2012). However, most researchers now agree that Aβ plaques are rather secondary events in the development of the disease (de Calignon et al., 2010; García-Sierra et al., 2003; Glass et al., 2010; Joseph et al., 2001; de la Torre, 2011; Lee et al., 2007; Rissman et al., 2004). Most importantly, the removal of Aβ by immunization did not ameliorate the progressive cognitive decline and did not improve survival of patients (Holmes et al., 2008). Neurofibrillary changes are thought to occur after changes in Aβ metabolism (Hardy and Selkoe, 2002).

A number of hypotheses regarding other possible underlying mechanisms of AD have been postulated. This review discusses recent findings regarding malfunctioning insulin signaling, dysfunction of mitochondria-associated membranes, cerebrovascular changes, increased oxidative stress and free radical formation, DNA damage, disturbed energy metabolism, and synaptic dysfunction, which are currently under discussion as potential disease-initiating causes of AD. Importantly, all of these processes are age-related and influenced by APOE-ε4. We will discuss whether they are present in very early stages of AD before clinical symptoms and neuropathology occur (see Figure 1). We will also point out when evidence suggests that the disturbances in the above mentioned processes have a link with Aβ metabolism. Changes in Aβ metabolism, although most likely not the primary cause of AD, may have an important impact on the progression of the disease process in AD. Our focus on soluble Aβ is supported by findings that soluble Aβ is highly neurotoxic and has been found to precede Aβ plaque formation (Leon et al., 2010). Furthermore, all genetic mutations leading to early onset AD have an effect on Aβ metabolism (Wu et al., 2012). It is thus possible that (subtle) changes in Aβ processing and production might be an important supporting factor for the development of full blown AD that is shared by the sporadic and inherited forms of AD. As AD is a complex, progressive disease, we will in the second part of this review discuss how the potentially disease initiating-factors may interact with each other.
Dysfunctional biological processes that contribute to Alzheimer Disease

Malfunctioning insulin signaling precedes MCI

Correct insulin signaling is important for cognitive function and memory. In neurons insulin stimulates growth, survival, differentiation, metabolism, protein synthesis, synapse formation, and plasticity (de la Monte, 2012). Insulin signaling has been found to be impaired in AD and in prodromal stages of the disease (Candeias et al., 2012). In the anterior frontal cortex at Braak stages II and III insulin, insulin receptor, insulin-like growth factor I (IGFI), IGFI receptor and IGFI gene expression are reduced compared to control tissue from Braak stages 0 and I (Rivera et al., 2005). Furthermore, insulin, IGFI and IGFI binding are already impaired in Braak stages II and III. In this brain area neuronal loss also positively correlates with diminishing insulin, IGFI and IGFI levels and their receptors. Type 2 diabetes mellitus (T2DM), which is characterized by peripheral insulin resistance and elevated fasting levels of insulin and glucose, is often accompanied by MCI (Gasparini et al., 2002). Talbot et al. (2012) reported in a post-mortem study that cognitive decline in AD patients is correlated with impaired insulin signaling in the hippocampus and the cerebellar cortex. Chronic peripheral hyperinsulinemia reduces insulin transport to the brain and thus leads to impaired brain insulin signaling (Neumann et al., 2008). In line with these results increased fasting plasma insulin levels and lower cerebrospinal fluid (CSF) insulin levels have been found in AD patients (Craft et al., 1998; Frölich et al., 1998; Roriz-Filho et al., 2009). However, others could not find differences in CSF insulin levels between control and AD subjects (Molina et al., 2002). It would be of interest to study insulin levels in the CSF of MCI patients to deepen our understanding of insulin signaling changes during the early stages of the disease.

Importantly, insulin and IGFI signaling regulate the expression and phosphorylation of tau protein (de la Monte, 2012): decreased insulin function leads to the over-activation of glycogen synthase kinase 3β (GSK-3β) which results in the hyperphosphorylation of tau, followed by tau-misfolding, neurofibrillary changes and consequently impaired cytoskeleton organization, synaptic failure and progressive neurodegeneration (Gasparini et al., 2001, 2002; de la Monte, 2012).

In addition to its influence on tau processing, insulin also affects Aβ homeostasis (see Figure 2): insulin and IGFI signaling stimulate the release of intracellular Aβ and thus reduce intraneuronal Aβ, promote tissue clearance of Aβ and protect neurons from Aβ-cytotoxicity by promoting the non-amyloidogenic pathway of AβPP processing (Di Carlo et al., 2010; Carro et al., 2002; Gasparini et al., 2001; Messier and Teutenberg, 2005; Solano et al., 2000). Accumulation of Aβ due to impaired insulin signaling further reduces insulin signaling, since Aβ interferes with insulin receptor autophosphorylation necessary for insulin signaling thereby causing a detrimental positive-feedback loop (Ling et al., 2002). Furthermore,
Aβ oligomers reduce the expression of insulin receptors and thus inhibit insulin signaling (Zhao et al., 2008). Supporting the influence of insulin signaling on Aβ and tau, Li et. al (2010) have shown that 3xTg mice with streptozotocin (STZ)-induced diabetes have increased Aβ, AβPP and tau levels compared to untreated 3xTg mice. In these mice the increased levels of Aβ, AβPP and tau were reduced by the stimulation of endogenous insulin release from β-cells.

Several studies targeting impaired insulin signaling and disturbed insulin levels have shown that elevation of central insulin levels has a positive effect on memory and general function in AD patients: In a randomized double-blind, placebo-controlled study in which MCI and AD patients received intranasal insulin treatment twice a day on 21 consecutive days, insulin administration improved
cognitive function, attention and functional ratings given by the caregivers (Reger et al., 2008). A recent study by Craft et al. (2012) in which AD patients received intranasal insulin daily for four months was able to replicate these findings. In line with these results the administration of an oral antidiabetic drug together with insulin in T2DM patients with mild-to-moderate AD prevented the decline of mini-mental state examination (MMSE) scores after 12 months of treatment (Plastino et al., 2010). These findings implicate that disturbed insulin levels and signaling might be an important target for the development of preventive AD therapies.

**Dysfunction of mitochondria-associated membranes may act as initiator of AD pathology**

More than 20 years ago, Vance (1990) described a membrane fraction that shared many but not all properties with the endoplasmatic reticulum (ER) and was isolated in association with mitochondria. These lipid raft-like mitochondria-associated membranes (MAM) form a physical bridge between the ER and mitochondria and are involved in the transfer of newly synthesized phospholipids between these cellular compartments (Browman et al., 2006; Fujimoto et al., 2012; Hayashi and Fujimoto, 2010; Vance, 1991). Interestingly, the cellular processes in which MAM are involved, i.e. mitochondrial function, cholesterol and phospholipid metabolism, and calcium homeostasis, have all been found to be altered in very early stages of AD (Camandola and Mattson, 2011; Pani et al., 2009; Schon and Area-Gomez, 2012; Su et al., 2010) suggesting a direct involvement of MAM in AD development (see Figure 1). Using subcellular fractionation, western blotting, γ-secretase activity assays, and immunocytochemistry, Area-Gomez et al. (2009) showed that PSEN1, PSEN2 and γ-secretase activity are abundantly present in MAM of both neuronal and non-neuronal cells, and are not homogenously distributed within the ER as has been assumed earlier (Chyung et al., 2005; Cupers et al., 2001; De Strooper et al., 1997). This finding suggests that γ-secretase cleavage of AβPP and thus the production of Aβ occurs at MAM.

Area-Gomez et al. (2012) recently showed that MAM activity and ER-mitochondria communication and connectivity are significantly increased in presenilin-mutant (PSEN1-/-, PSEN2-/- and PSEN1/PSEN2-/-) mouse embryonic fibroblasts and in fibroblasts from patients with familial or sporadic AD. This not only indicates that PSEN function forms a negative feedback mechanism for MAM activity, but it also proposes dysfunctional MAM as common denominator of sporadic and familial AD. Most importantly, it shows that the increased production of Aβ in AD might be due to overactive MAM (see Figure 2).

Alterations in cholesterol and lipid composition due to abnormal MAM function might further influence the cleavage of AβPP by γ-secretase and the
amount and composition of Aβ. AβPP processing and release of Aβ at the MAM provides an explanation how Aβ accumulates in mitochondria and contributes to mitochondrial dysfunction and subsequent elevation of oxidative stress (Atamna and Frey, 2007; Hardy and Selkoe, 2002; Yan et al., 2012). Interestingly, it has been reported that AβPP processing and Aβ production and clearance is mediated by gangliosides and sphingomyelin lipids, both abundantly present in MAM (Grimm et al., 2012; Yuyama and Yanagisawa, 2010) and reduced in brains of AD patients (Crino et al., 1989; Pernber et al., 2012).

Cerebrovascular changes precede cognitive decline in AD
Cerebrovascular changes such as decreased vascular density, enhanced vessel curvature, degeneration of smooth muscle cells in the vessel walls, alterations of the vascular endothelium, capillary fragmentation and abnormal permeability of the blood brain barrier are common pathological features seen in brains of AD patients (Fischer et al., 1990; Kalaria, 1996, 2002; Perlmutter, 1994). Importantly, they precede cognitive decline and become more pronounced with disease progression (Nicolakakis and Hamel, 2011). Studies show that cerebrovascular lesions are significantly more frequent in autopsy-confirmed AD patients than in age-matched controls and that incidence and severity of cerebrovascular lesions are positively correlated with Braak stages of AD patients (Jellinger, 2010; Nicoll et al., 2004). Moreover, capillary length is greatly reduced in AD patients (-60% compared to controls) (Wu et al., 2005) and silent brain infarcts, a pathology seen frequently in healthy elderly individuals, more than double the risk of dementia (Vermeer et al., 2003, 2007).

There is evidence, that Aβ production takes place in perivascular cells (Wisniewski et al., 2000) and in smooth muscle cells of brain capillaries (Frackowiak et al., 2003), where it either accumulates or is secreted (see Figure 2). Low-density lipoprotein receptor related protein-1 (LRP1), the main Aβ clearance receptor, is abundantly expressed in the smooth muscle cells of the cerebrovasculature and plays a key role in the removal of Aβ from the brain via the blood brain barrier into the plasma (“peripheral Aβ sink mechanism”) in healthy subjects (Deane et al., 2009). Kanekiyo et al. (2012) showed that AβPP/PS1 mice with a conditional deletion of the Lrpl gene in vascular smooth muscle cells had higher Aβ levels and developed much stronger Aβ plaque deposition and CAA compared to control mice with normal Lrpl expression. Lrpl expression is regulated by the transcription factor mesenchyme homeobox 2 (Mox2), which is involved in angiogenesis, vascular differentiation and apoptosis (Wu et al., 2005). MEOX2 levels are reduced in brains of AD patients at Braak stage V/VI, which causes reduced LRP1 expression levels at vessels and impaired Aβ clearance (Donahue et al., 2006; Wu et al., 2005). Importantly, chronic cerebral hypoperfusion and ischemia
induce pro-apoptotic caspase-3 activation as part of the intrinsic and extrinsic apoptosis pathway which not only leads to neuronal cell death (Parikh et al., 2003), but also suppresses the expression of the BACE1-trafficking molecule ADP-ribosylation factor-binding protein (GGA3) (Broughton et al., 2009). In turn, BACE1 is stabilized, favoring ß-amyloidogenic processing of AßPP by increased BACE1 activity. In line with these results, chronic hypoxia results in significantly elevated Aß plaque deposition and worsened cognitive function in 8 month-old AßPP23 mice due to enhanced BACE1 transcription and expression independent of caspase-3 activation (Sun et al., 2006). Furthermore, cerebral ischemia has also been shown to accelerate AßPP expression in rodents (van Groen et al., 2005; Wen et al., 2004). Importantly, also in the human cortex and hippocampus ischemic and hypoxic insults induce a marked increase in Aß expression (Jendroska et al., 1995; Qi et al., 2007; Wiśniewski and Maślińska, 1996). Additionally, Zetterberg et al. (2011) reported that serum levels of Aß42 are elevated in humans after hypoxia due to cardiac arrest, while Bibl et al. (2012) found strongly decreased Aß40 plasma levels after acute stroke. Supporting the rodent data, also in humans BACE1 levels were found to be elevated after stroke in patients that developed cognitive impairment (Qian et al., 2012).

Besides accelerating Aß production, hypoxia also inhibits Aß breakdown by decreasing the expression and activity of several Aß-degrading enzymes like neprilysin and endothelin-converting enzyme (EDN) (Nalivaevaa et al., 2004; Wang et al., 2011). Tg2576 AD mice were found to have increased microvasculature expression of hypoxia-associated genes, like matrix metalloproteinase 2, an enzyme involved in breakdown of extracellular matrix and tissue remodeling, and caspase-3 and decreased expression of the anti-apoptotic protein Bcl-xL (Grammas et al., 2011). Significant hypoperfusion as assessed by pulsed arterial spin-labeling perfusion MRI was also found in patients with MCI and AD (Johnson et al., 2005). Deposition of Aß in the leptomeningeal and cerebral blood vessel walls (cerebral amyloid angiopathy; CAA), caused by efflux of Aß from the parenchyma to the periphery, is observed in both AD patients and AD mouse models (Dorr et al., 2012) and causes degeneration of vascular smooth muscle cells, impairment of angiogenesis and vascular tone, and decreased cerebral blood flow (Miao et al., 2005; Shin et al., 2007). These pathological changes ultimately lead to chronic cerebral hypoperfusion and cerebral ischemia of the brain tissue innervated by the affected capillaries, resulting in hypoxia and finally neuronal death (Pfeifer et al., 2002; Rensink et al., 2003).

Importantly, Aß deposition and hypoxic ischemia have been shown to have a detrimental synergistic effect on cognitive function and neuronal death (Iwasaki et al., 2006). Mice overexpressing a human wild-type AßPP gene were more susceptible for infarcts by middle-cerebral artery occlusion, a model for cerebral
hypoperfusion and hypoxia, than control mice (Koistinaho et al., 2002). Interestingly, the enhanced vulnerability was not due to altered cerebral blood flow, but associated with increased activation of microglia and could be restored by anti-inflammatory treatment with aspirin or a selective p38 mitogen-activated protein kinase (MAPK) inhibitor, indicating that a pro-inflammatory environment can accelerate vulnerability to cerebrovascular changes and hypoxia.

Pathological cerebrovascular changes are likely to contribute to the development of AD as they have a clear influence on cerebral ischemia and Aβ production and clearance (see Figure 2). The influence of cerebrovascular changes on insulin signaling will be discussed later in this review.

**Increased oxidative stress and free radical formation**

Due to relatively low levels of antioxidants and the high oxygen consumption rate, the brain is extremely vulnerable to free radical-induced damage (Coyle and Puttfarcken, 1993). Elevated oxidative stress and free radical levels are one of the earliest observed changes in AD pathology, already present in MCI patients (see Figure 1; Aluise et al., 2011; Barone et al., 2011; Butterfield et al., 2006; Jomova et al., 2010; Keller et al., 2005; Liochev and Fridovich, 1999; Markesbery et al., 2005; Smith et al., 1996; Sultana et al., 2010) and are known to cause damage to proteins, lipids, nuclear and mitochondrial DNA as well as to RNA. DNA damage will be discussed in more detail later in this review.

Accelerated oxidative stress and increased formation of free radicals are generally thought to precede Aβ plaque formation (Frederikse et al., 1996; Yan et al., 1995). A post-mortem study of AD and non-demented control cases found that oxidative damage was greatest in early phases of the disease but decreased with disease progression, i.e. inversely correlated with Aβ plaque burden and duration of dementia suggesting that AD is associated with compensatory changes that reduce damage from reactive oxygen (Nunomura et al., 2001). Isoprostanes, sensitive markers of lipid peroxidation in vivo, were significantly higher in cerebral and hippocampal brain homogenates, urine and blood plasma of Tg2576 transgenic mice compared to wild-type mice from 8 months of age onwards whereas Aβ plaque deposition and elevated Aβ40 and Aβ42 levels were only present from 12 months of age onwards indicating that oxidative stress is an upstream events in AD pathology (Praticò et al., 2001). Studies in humans showed that isoprostane levels in the CSF are elevated in patients with preclinical AD (Montine et al., 2010), MCI (Montine et al., 1999) and AD (Montine et al., 1998; Praticò et al., 2000) and that they increase with disease duration (Quinn et al., 2004). Isoprostane levels correlate with CSF tau and Aβ levels, MMSE scores and the number of APOE-ε4 alleles (Duits et al., 2013; Praticò et al., 2000).
However, other studies suggest that oxidative stress levels are not elevated in preclinical AD [140] when Aβ plaques are present and that Aβ has a role as free radical-inducing agent. Aβ was found to stimulate the generation of free radicals indirectly by damaging mitochondria and directly by regulating the redox activity of several metals such as iron and copper (Manczak et al., 2006; Reddy, 2006; Smith et al., 2010). By using multiphoton microscopy McLellan et al. (2003) showed a direct association between deposition of Aβ and free radical formation in vivo in AD transgenic mice (Tg2576 and PDAPP) and ex vivo on post-mortem brain tissue from AD patients. These findings support a relationship between increased oxidative stress levels and changes of Aβ levels along with the progression of the disease. Yet, it is still controversial whether an increase of oxidative stress and free radicals constitute a cause or consequence of Aβ pathology (see Figure 2).

Metal homeostasis has been found to be severely disturbed in brains of AD patients, characterized by intracellular accumulation of iron and extracellular deposition of zinc and copper in Aβ plaques (Bush, 2012). Disturbances in the zinc transporters 1, 3, 4, and 6 have been reported to already occur in preclinical AD (Bossers et al., 2010; Lyubartseva et al., 2010) indicating that disturbances in zinc homeostasis may contribute to AD pathology. Iron-accumulation in the brain is a source for redox-generated free radicals, increases with disease progression and has been shown to co-localize with NFTs and Aβ plaques in post-mortem brains samples of patients with AD and MCI (Smith, 1997; Smith et al., 2010). It is hypothesized that Aβ-mediated trapping of metals is responsible for the local reduction of metals at the synapse which results in increased free radical formation, disturbed synaptic transmission and cognitive decline (Bush, 2012; Hayashi et al., 2007). These findings indicate that the formation of free radicals is partly caused by a disturbance in metal homeostasis secondary to Aβ accumulation and plaque formation.

Accelerated free radical and oxidative stress production is an early event in AD. The increase of oxidative stress levels in mitochondria found in AD brains may be a cause (Liu et al., 2012; Manczak et al., 2006) or consequence (Yan et al., 2012) of mitochondrial dysfunction. Interaction of oxidative stress, mitochondrial dysfunction, Aβ accumulation and perturbed metal homeostasis might lead to a vicious circle which stimulates the progression of the disease.

**DNA damage occurs in patients with MCI**

Researchers have suggested that DNA damage contributes to the development of AD and other neurodegenerative diseases (Brasnjevic et al., 2008; Moreira et al., 2008). Important support for this idea was recently reported. Genetic ablation of the DNA repair enzyme endonuclease VIII-like1 (NEIL1) causes learning and memory deficits in mice (Canugovi et al., 2012) and DNA modifications and defective DNA
repair are important in neurodegenerative and cognitive disorders (Day and Sweatt, 2011; Gräff et al., 2011; Rass et al., 2007). Increased DNA damage (Wang et al., 2006) and reduced DNA repair capacity (Shackelford, 2006) have been reported in post-mortem tissue from patients with MCI (Braak stage III; see Figure 1). Furthermore, it is long known that increased DNA damage occurs in later stages of AD (Mecocci et al., 1994; Mullaart et al., 1990; Myung et al., 2008). Watabe-Rudolph et al. (2012) found increased activity of the enzyme chitinase, a biomarker of DNA damage, but also activated microglia and macrophages in inflammatory processes (Correale and Fiol, 2011), in the CSF of AD patients.

Very recently the first clear link between DNA damage and Aβ pathology was established. Suberbielle et al. (2013) reported that AβPP transgenic mice showed more neuronal DNA double-strand breaks and diminished DNA repair compared to wild-type mice after exploring a novel environment. In the same study they found Aβ-induced DNA damage in neuronal cultures (Figure 2).

**Disturbed energy metabolism occurs in patients with MCI**

Deranged energy metabolism is a hallmark of AD and already present in patients with MCI and very early AD (Hoyer, 2004; Kapogiannis and Mattsion, 2011; Mamelak, 2012; Mosconi, 2005). The main energy source in the brain comes from the oxidation of glucose leading to the production of adenosine triphosphate (ATP) (Mosconi et al., 2008). Changes in glucose utilization therefore ultimately affect central energy metabolism necessary for physiological brain functioning. Tau-O-GlcNAcylation, a post-translational modification of tau protein which is regulated by glucose metabolism, negatively correlates with tau hyperphosphorylation (Liu et al., 2009). A reduction in glucose metabolism therefore leads to the formation of NFTs by a decrease in tau-O-GlcNAcylation. In a post-mortem study, Bubber et al. (2005) found a decrease of mitochondrial enzymes involved in cerebral energy metabolism, such as pyruvate, isocitrate and α-ketoglutarate dehydrogenases with AD disease progression. Furthermore, Salehi and Swaab (1999) found a clear reduction in brain metabolism in several severely affected brain regions including the nucleus basalis of Meynert, the hippocampus and the thalamus as determined by the size of the Golgi apparatus. However, they could not find a relation between the reduction in brain metabolism and plaques or tangles. Additionally to the impaired energy supply, reduced ATP levels might contribute to excitotoxicity due to accumulation of intracellular sodium ions as a result of a dysfunctional Na2+/K+-ATPase (Parihar and Brewer, 2007).

Since studies in post-mortem tissues are limited to indirect measurements of energy metabolism such as expression of genes involved in energy metabolism, functional neuroimaging techniques like 2-deoxy-2-[18F]fluoro-D-glucose (FDG) positron emission tomography (PET) scans and functional magnetic resonance
imaging (fMRI) are used to provide a more direct measure of changes in central energy metabolism. It was found that the decrease in cerebral metabolic rate for glucose correlated with the topography and severity of AD pathology (Mosconi et al., 2008). In a FDG-PET study by Fouquet et al. (2009) metabolic decrease in several brain regions was assessed in MCI patients who, after a follow-up period of 18 months did or did not develop AD based on NINCDS-ADRDA Alzheimer’s criteria (McKhann et al., 1984). Patients that did develop AD were found to have a significantly stronger decrease in energy metabolism (defined as the percent annual change of the PET scan) in both, the subgenual and anterior cingulate cortex compared to patients that did not develop AD, indicating that metabolic decrease is a strong risk factor for AD. Thus, PET-monitored decrease in energy metabolism might be a useful marker for the progression of AD.

Dysfunctional energy metabolism seems to play an important role in the development of AD as it affects both Aβ and tau pathology (Liu et al., 2012) and correlates with the severity of the cognitive decline. We will discuss the effects of decreased energy supply of the brain on synaptic dysfunction and elevated oxidative stress levels later in this review.

**Synaptic changes precede Aβ pathology**

Changes in synapse function are a characteristic hallmark of AD pathology already present at early stages of the disease and in patients with MCI, and before Aβ plaques or neurofibrillary changes are visible (Masliah et al., 2001; Scheff et al., 2007). Importantly, synaptic loss in the hippocampus and neocortex is the major structural correlate of cognitive decline in AD (Coleman et al., 2004; DeKosky et al., 1996; Scheff and Price, 2006; Terry et al., 1991). Several post-mortem studies showed that the progressive reduction in synapse number in brain samples of individuals with MCI or AD compared to individuals without cognitive decline positively correlates with MMSE scores (DeKosky and Scheff, 1990; Scheff et al., 2006; Sze et al., 2000). However, no correlation between Braak stage and synaptic loss was observed. This finding is surprising inasmuch a reduction in the MMSE score reflects a reduction in cognitive function, and cognitive function does correlate with Braak stage classification of AD patients (Braak et al., 2006). Indeed various other studies reported a close correlation between reduction in synaptic markers and the amount of neurofibrillary tangles (Heffernan et al., 1998; Masliah et al.). Hypotheses on the relationship between synaptic loss and the development of neurofibrillary changes differ among researchers. Several authors hypothesize that synaptic dysfunction precedes the formation of neurofibrillary changes (Masliah et al., 1992; Yoshiyama et al., 2007), whereas others observed synaptic loss induced by neurofibrillary events both in vitro (Hall et al., 2000; Thies and Mandelkow, 2007), and in vivo (Hoover et al., 2010; Schindowski et al., 2006).
A close relationship exists between a rise in soluble Aβ and the development of synaptic dysfunction (Klyubin et al., 2012; Nimmrich and Ebert, 2009; Selkoe, 2002; Shankar and Walsh, 2009). Binding of soluble Aβ oligomers to synaptic membranes is highly synaptotoxic as it leads to increased oxidative stress, reduction in dendritic spines and decreased translocation/increased internalization of synaptic receptors (Gong et al., 2003; Klein et al., 2004; Lacor et al., 2004). Moreover, acute neuronal overproduction of Aβ blocks synaptic long-term plasticity and reduces synaptic contacts in hippocampal slices (Kessels et al., 2010; Wei et al., 2010). Mucke et al. (2000) found that synaptophysin-immunoreactivity was significantly decreased in mice expressing either human AβPP (hAβPP) harboring different mutations (hAβPP695, hAβPP751, and hAβPP770) or functional human AβPP although the latter did not show Aβ plaque deposition, implicating that Aβ-induced synaptotoxicity is plaque-independent. Supporting this view, Tomiyama et al. (2010) reported on an AβPP transgenic mouse which develops intraneuronal Aβ accumulations without plaques and shows impaired synaptic plasticity and memory function. These findings are in line with a study by Shankar et al. (2008) in which mouse hippocampal brain slices were incubated with the soluble fraction of Aβ oligomers, isolated from the cerebral cortex of patients with AD. The study revealed a significant decrease in long-term potentiation (LTP), a key mechanism in learning and memory, increased long-term depression (LTD), which selectively weakens synaptic connections, and reduced dendritic spine density. Furthermore, the injection of Aβ fraction extracted from human brain tissue caused a disruption of a learned passive avoidance behavior in rats (Shankar et al., 2008). Consequently, soluble Aβ not only causes synaptic loss and deregulation of synaptic plasticity on a molecular and functional level but also impairs memory consolidation.

In the last years evidence has arisen that very early stages of MCI/AD are accompanied by neuronal hyperexcitability (Celone et al., 2006; Dickerson et al., 2004, 2005; Hämäläinen et al., 2007; Kircher et al., 2007). Elderly with AD more often suffer from epileptic seizures than cognitively intact individuals (Palop and Mucke, 2009). Several lines of transgenic AD mice have spontaneous seizures (Minkeviciene et al., 2009; Palop et al., 2007) or increased seizure activity in response to excitotoxic challenge (Palop et al., 2007; Del Vecchio et al., 2004; Westmark et al., 2008). In the hippocampus this hyperexcitability may alter inhibitory circuits which may result in deficits in hippocampal-based memory tasks (Palop et al., 2007). In line with this idea Bakker et al. (2012) recently showed that a low dose of the antiepileptic drug levetiracetam reduced hippocampal hyperactivity in patients with MCI and improved their memory performance. Sanchez et al. (2012) could show the same positive effect in an AβPP transgenic mouse. Increased synaptic activity also directly promotes the production and
secretion of Aβ (Cirrito et al., 2005; Kamenetz et al., 2003) opening the possibility that neuronal hyperactivity precedes Aβ accumulation (see Figure 2) and once Aβ is accumulated it acts as negative feedback on neuronal activity.

Recent studies suggest that synaptic dysfunction occurring in AD is the consequence of a failing adaptation process, which is reflected by a rise of synaptic markers in the early phase of the disease and a decrease at later stages (Arendt, 2009). In line with this hypothesis, we recently found a significant increase in gene expression associated with synaptic plasticity, synaptic transmission, exocytosis of neurotransmitter and voltage-gated potassium channels (essential for the generation of action potentials) in the prefrontal cortex of patients at a very early disease state (Braak stage II) before neuropathological and neuropsychological changes were observed, followed by a decreased expression of those genes later in AD (Braak stage III-VI) (Bossers et al., 2010). Interestingly, we also found an increase in intraneuronal Aβ from Braak stage I to III, followed by a decrease in Braak stage IV-VI. Among the first up- and then downregulated genes we found the zinc transporter ZNT3, which is responsible for the transport of zinc into the glutamatergic synapse where zinc maintains synaptic transmission (Adlard et al., 2010). In line with our results, Bjorklund et al. (2012) also reported decreased ZNT3 levels in patients with AD. Cognitively healthy subjects with AD pathology, however, did not have decreased ZNT3 levels indicating that ZNT3 mediated zinc storage in the presynapse can reduce free zinc levels at the postsynapse and thereby prevent the binding of Aβ oligomers and consequently cognitive decline. Zinc is also involved in the gene expression regulation of several synaptic proteins (Sensi et al., 2011). These findings indicate, that synaptic dysfunction observed in AD is preceded by increased synaptic activity and plasticity in very early disease stages, possibly in response to elevated levels of soluble Aβ (see Figure 2), whose toxic action might partly be mediated by disturbances in metal homeostasis.

The question that remains to be answered is what causes the increased generation of Aβ oligomers in early stages of AD. Aβ oligomers are produced by β-secretase 1 (BACE1) and γ-secretase-mediated cleavage of AβPP (Dietrich et al., 2010). Zhong et al. (2007) found a significant elevation of both BACE1 levels and activity in the CSF of patients with MCI compared to both AD and controls. Therefore, a trigger exists that induces increased BACE1 expression and activity prior to AD, which disappears with progression of the disease. Since a variety of factors like aging, ischemia, the increase of several cytokines and free radicals that can induce a rise in BACE1 levels are all present in early AD, there is no consent regarding the cause for the increase of BACE1 levels and activity until now (Fukumoto et al., 2004; Heneka et al., 2005; Hong et al., 2003; Tamagno et al., 2002, 2005). However, many researchers agree that reduction of BACE1 levels might ameliorate the progression of AD (Barman and Prabhakar, 2012; Wen et al., 2004; Zhang, 2012). The decreased
expression of BACE1 using small interference RNA in the hippocampus of a transgenic mouse model for AD (human AβPP751; V717I; K670M/N671L) was able to reduce Aβ levels, ameliorate neurodegeneration and reverse behavioral deficits (Singer et al., 2005). Since several years, research aims to develop a safe and clinically applicable BACE1 inhibitor for clinical trials in humans (Baxter et al., 2007; Cumming et al., 2012). Very recently, the BACE1 inhibitor MK-8931 entered a phaseII/III clinical study (EPOCH study) in patients with mild-to-moderate AD (ClinicalTrials U.S. National Institutes of Health, 2012). Other BACE1 inhibitors have been developed recently and showed promising results in mice (May et al., 2011; Sankaranarayanan et al., 2008).

In conclusion, changes in synaptic function can be detected in very early stages of the disease, which might be either the cause or the consequence of increased levels of highly synapto- and neurotoxic soluble Aβ. We will discuss the influence of insulin and energy metabolism on synaptic function later in this review.

**Interactions of potential causes of Alzheimer Disease**

Above we reviewed potentially causative molecular events in AD. These events show a correlation with the severity of the disease at the functional and/or structural level, and are present at very early or even prodromal stages of AD. We will now discuss the relation of these biological processes with the two main risk factors for sporadic AD, aging and APOE-ε4. Furthermore, we will discuss how they may interact with and influence each other (see Figure 2). It is very important to keep in mind that it is possible that different processes act as AD-initiating factors in distinct groups of patients. One patient population, e.g. those with obesity might show initial disturbances in insulin signaling, while others, e.g. those with cardiovascular disorders, show changes in their vasculature.

**Interaction with the main risk factors aging and APOE-ε4**

All dysregulated biological processes that we have reviewed as potential AD-initiating factors are related to age, the main risk factor for AD, providing a possible role for aging itself as initiating event in the development of AD. Insulin signaling and sensitivity are very often decreased in the elderly (Chang and Halter, 2003). In line with this the prevalence rates of diabetes increase with age (CDC, 2011). Additionally, aging restricts cell cycle entry and thus the regeneration of β cells, which are crucial for insulin secretion (Kushner, 2013). Key mitochondrial functions, namely increased ROS generation and enhanced levels of oxidative stress, reduced ATP supply, and activation of apoptosis, are associated with aging (Conley et al., 2007; Seo et al., 2010). Furthermore, it has long been recognized that changes in the function and structure of the vasculature are also age-related and can lead to diminished cerebral blood flow (Marín, 1995). Modifications and
damage of DNA accumulate during aging and contribute critically to the aging process (Bohr et al., 1998; Hamilton et al., 2001; Lu et al., 2004; Rutten et al., 2003, 2007). Moreover, changes in energy regulation occur during normal aging including a decrease of energy expenditure (Roberts and Rosenberg, 2006) and a decline in glucose metabolism (Salmon, 2012). Other characteristic hallmarks of aging are altered synaptic functions including LTP, altered synaptic morphology, synapse loss and a loss of synaptic protein expression (Burke and Barnes, 2006; Driscoll et al., 2006; McGahon et al., 1999; Ojo et al., 2012; Rosenzweig and Barnes, 2003).

All of the reviewed biological processes are also influenced by the main genetic risk factor for sporadic AD, APOE-ε4. A number of studies have linked APOE-ε4 and insulin metabolism. Peila et al. (2002) found that the risk of AD is highest amongst APOE-ε4 carriers with T2DM and that the highest number of AD lesions is present in brain tissue of ε4 diabetic patients. In addition low CSF to plasma insulin ratios in AD patients are influenced by APOE genotype (Craft et al., 1998). The administration of insulin via peripheral (Craft et al., 2000) and intranasal (Reger et al., 2006) routes also improved cognition and these effects were more prominent in non-APOE-ε4 carriers. APOE-ε4, furthermore, causes mitochondrial dysfunctions (Chang et al., 2005; Devi et al., 2006) and impaired lipolytic processing (Li et al., 2013) suggesting that APOE-ε4 may be an influential factor of MAM function. Moreover, the APOE-ε4 allele increases the risk for vascular changes (Anand et al., 2009; Caselli et al., 2011; Elosua et al., 2004; Nicoll et al., 2004; Yip et al., 2005). In addition, it has the lowest protective activity against oxidative DNA damage compared to other APOE isoforms (Tamaoka et al., 2000). APOE-ε4 carriers have been shown to have decreased activity in the hippocampus and cortex during cognitive tasks (Lind et al., 2006; Trivedi et al., 2006) which is in line with decreased glucose metabolism in APOE-ε4 carriers (Jagust and Landau, 2012). A link between decreased neuronal metabolic rate and APOE-ε4 allele dose has been reported (Dubelaar et al., 2004; Reiman et al., 2001, 2004, 2005; Salehi et al., 1998). Others showed that healthy APOE-ε4 carriers had hyperactive brain regions during cognitive tasks (Bondi et al., 2005; Bookheimer et al., 2000; Fleisher et al., 2005; Wishart et al., 2006). Even though the latter findings are in conflict with the findings from Trivedi et al. (2006) and Lind et al. (2006), they support the idea that APOE-ε4 also influences synaptic activity. Indeed, we could also show that APOE-ε4 carriers showed increased expression levels of synaptic genes at Braak stages III and IV (Bossers et al., 2010).

Insulin signaling interacts with MAM function, cerebrovascular changes and energy metabolism
As discussed earlier, gangliosides and sphingomyelin regulate AßPP processing and Aß production and clearance, and those lipids are abundantly present in MAM
(Grimm et al., 2012; Yuyama and Yanagisawa, 2010). Interestingly, gangliosides and sphingomyelin have also been implicated in the regulation of insulin signaling and sensitivity. Heterozygous sphingomyelin synthase 2 (Sms2) knock-out mice show increased insulin sensitivity compared to control mice (Li et al., 2011). Moreover, it is possible that deranged lipid compositions induced by insulin dysfunction affect the lipid raft-like MAM structure and function (Baron, 2002). Malfunctioning insulin signaling is associated with increased cholesterol levels and cholesterol is essential in MAM function (see Figure 2). Depletion of cholesterol leads to an enhanced interaction between MAM and mitochondria in vitro (Fujimoto et al., 2012), reinforcing the assumption that significantly elevated cholesterol levels might weaken the association between MAM and mitochondria and thereby contribute to dysfunction of both mitochondria and MAM. It has been hypothesized that perturbed cholesterol homeostasis might affect AβPP cleavage by γ-secretase in the MAM and thereby contribute to deregulated Aβ production (Schon and Area-Gomez, 2012).

Insulin dysfunction stimulates pathological cerebrovascular changes (see Figure 2), both indirectly via the aforementioned stimulation of Aβ production and inhibition of Aβ clearance resulting in accumulation of Aβ in the vessel walls and deranged lipid homeostasis, as well as directly via its role as vasoactive hormone (Baron, 2002; Murray et al., 2011). Salkovic-Petrisic et al. (2011) found vascular Aβ depositions in the brains of the intracerebroventricular STZ-Wistar rats starting 3 months after STZ treatment and progressing to profound CAA 6 to 9 months after STZ treatment. Insulin signaling is essential in endothelial nitric oxide-dependent vasodilation, thereby directly affecting blood flow and vascular tone, features which are altered in AD patients (Murray et al., 2011). Deranged insulin function is also associated with elevated levels of free fatty acids, further exacerbating the impairments in glucose uptake on the one hand and causing vascular endothelial dysfunction by inhibiting release of nitric oxide on the other hand (Baron, 2002). We reviewed findings on cerebrovascular changes in AD and the role of LRP1 above. Importantly, LRP1 has recently been shown to also be involved in insulin signaling (Liu et al., 2011). Conditional Lrp1 forebrain knock-out mice had 2-fold higher insulin levels at 12 months of age compared to control mice, and glucose and insulin tolerance were markedly reduced. These findings provide a direct link between insulin dysfunction and cerebrovascular changes in AD pathology. Proper insulin function is essential for energy metabolism and glucose homeostasis in the brain. Thus, impaired insulin signaling directly affects and disturbs energy homeostasis by inhibiting glucose uptake via insulin-dependent glucose transporters GLUT1 and GLUT4 present in the brain, especially in the hippocampus (Craft and Watson, 2004; Zhao et al., 1999). Neurons have a limited glycolytic capacity (Yan et al., 2012), making them extremely vulnerable to
impaired glucose supply. Furthermore, insulin signaling affects glucose utilization indirectly by inhibiting noradrenaline uptake, which subsequently blocks the availability of glycogen stores in glia cells (Moreira, 2012). Recurrent moderate hypoglycemia in STZ-diabetic rats causes microglia activation, cognitive impairment and hippocampal thinning (Won et al., 2012). Alterations of glucose metabolism by starvation or intraperitoneal injections of insulin caused hyperphosphorylation of tau in the hippocampus and cerebral cortex of C57BL/6NJcl mice similar to the events seen in brains of AD patients (Planel et al., 2004).

In summary, insulin signaling affects the formation of neurofibrillary changes and Aβ plaques, MAM function, cerebrovascular changes and glucose metabolism (see Figure 2). This implies an important role for disturbances in insulin homeostasis for the development of AD.

**Oxidative stress interacts with DNA damage and energy metabolism**

The role of oxidative stress in early stages of AD has been discussed above. Oxidative stress causes DNA damage (Ismail and Hendzel, 2008; Lu et al., 2004). Moreover, many studies suggest that increased oxidative stress causes dysfunction in energy metabolism (Mamelak, 2012; Nunomura et al., 2001). Oxidative stress hinders glucose utilization by stabilizing the transcription factor hypoxia-induced factor 1α (HIF1α), followed by a reduction in glucose transporter expression and abolished insulin signaling (Mamelak, 2012). Elevated levels of oxidative stress cause a redirection of carbohydrates (glucose) towards the pentose phosphate pathway (PPP), resulting in a down-regulation of glycolysis and oxidative phosphorylation (Martins et al., 1986; Palmer, 1999). In line with these findings, glucose-6-phosphate dehydrogenase, the initiating enzyme of the PPP, is significantly elevated in brains of MCI and AD patients (Soucek et al., 2003; Sultana et al., 2008). Furthermore, it has been found that several enzymes essential in glucose metabolism are oxidatively modified and thereby inhibited in AD brains, leading to repression of glucose utilization and ATP production which indicates a direct relationship between deranged energy metabolism and oxidative stress (Sultana et al., 2006). However, oxidative stress cannot only be a cause for the disruption of energy metabolism but also a consequence (see Figure 2): A decrease in glucose utilization impedes the electron transport chain which results in deregulation of the cellular calcium homeostasis and increased ROS production (Lambert et al., 1998). Therefore, it is possible that once energy metabolism is disturbed or oxidative stress occurs, a feed-forward cascade establishes, favoring further derangements.
Synaptic function interacts with insulin signaling, energy metabolism and DNA damage

Synaptic function is influenced by insulin signaling (see Figure 2). Insulin receptors are densely present at synapses and are part of postsynaptic densities, which concentrate neurotransmitter in the synaptic cleft to provide a close contact between presynaptic neurotransmitter releasing sites and postsynaptic receptors (Ziff, 1997). Considering this synaptic distribution of insulin receptors, it is likely that proper insulin function contributes to synaptic transmission (Gerozissis and Kyriaki, 2003). Furthermore, intact insulin signaling is obligatory for neuronal growth, differentiation, and function: It regulates the expression and translocation of γ-aminobutyric acid (GABA) receptors, the most important inhibitory receptor in the CNS, to the plasma membrane and stimulates their expression in postsynaptic neurons (Laron, 2009; Wan et al., 1997). Moreover, Shonesy et al. (2012) studied the effect of impaired insulin function on glutamatergic synaptic physiology using the intracerebroventricular STZ Wistar rat model and found that impaired central insulin function causes profound inhibition of hippocampal basal synaptic transmission and LTP, which correlated with decreased expression of NMDA receptor subunits NR2A and NR2B. Brain derived neurotropic factor (BDNF) and stargazin, an AMPA receptor auxiliary subunit, both mediating the translocation of AMPA receptors to the postsynaptic membrane were decreased as well.

Interestingly, de Felice et al. (2008) reported that insulin has a protective function against Aβ oligomer-induced synaptic damage. Upon exposure to Aβ oligomers, a profound down-regulation of insulin receptors in plasma membranes of primary hippocampal neuronal cultures was observed before loss of dendritic spines occurred. This Aβ oligomer-induced reduction of insulin receptors, elevation of oxidative stress and deterioration of dendritic spines was completely prevented by pre-incubation of neurons with insulin. Rosiglitazone, an insulin-sensitizing drug used for treatment of T2DM, potentiated the effect at submaximal doses of insulin. The protective effect of insulin is due to an alteration of the Aβ oligomer binding sites on the plasma membrane, mediated by insulin receptor downstream signaling.

Liu et al. (2012) recently showed that restoring the deficits in energy metabolism improved cognitive function, Aβ plaque and tau pathology, mitochondrial integrity and increased levels of several proteins involved in synaptic plasticity and neuronal survival in 3xTgAD mice. These findings indicate a potential link between very early changes in cortical energy metabolism and later occurring synaptic dysfunctions in AD. Further supporting evidence comes from the finding that a disturbance in glucose metabolism leads to reduced activity of the pyruvate dehydrogenase complex and acetylcholine transferase, which in turn results in decreased production and availability of neurosteroids and acetylcholine, ultimately leading to impaired synaptic function (Hoyer, 2000).
Most interestingly, blocking neuronal hyperactivity in AβPP transgenic mice not only improved learning and memory in these mice, but also normalized increased levels of DNA double-strand breaks (Suberbielle et al., 2013). This indicates that aberrant synaptic activity, resulting from increased Aβ levels, exacerbates DNA damage (see Figure 2).

Conclusion

Based on the reviewed findings, we propose the following model/hypothesis. Early dysfunctions in insulin signaling, synaptic and cerebrovascular changes and deranged MAM function precede MCI. The disturbances in these age and APOE-ε4 related processes appear to interact with each other and this leads to the development of the classical neuropathological hallmarks of AD, namely the formation of plaques and tangles (Figure 2). It is not clear, however, how the changes in the above mentioned processes result in full blown AD. The available evidence suggest a central role for β-amyloidogenic AβPP processing. Aβ plaque deposition and impaired Aβ-clearance are directly affected by changes in the above mentioned processes. Increased Aβ levels, however, can also influence these events. Changes in AβPP metabolism and the primary dysregulated biological processes can lead to an imbalance in free radical formation and oxidative stress, DNA damage, impaired energy metabolism, and synaptic dysfunction and loss, followed by neurodegeneration. Future research should aim at understanding how changes in either of these processes affect the other and in which patient populations they might act as disease-initiating events in order to identify possible molecular markers and new targets for AD therapy.
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