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Oxidative Stress, DNA Damage and Repair Pathways in Patients with Type 2 Diabetes Mellitus

*Jessica E.B.F. Lima, Danilo J. Xavier
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Abstract

Type 2 diabetes mellitus (T2D) is characterized mainly by insulin resistance and/or deficiency, presenting risk factors related to aging, hypercaloric diet and sedentary lifestyle. Hyperglycemia, a hallmark of T2D, contributes significantly to the production of reactive oxygen species (ROS), inducing oxidative stress and various cellular and molecular changes in the body. As a consequence, several signaling pathways may be affected, mainly involving biological processes such as inflammation, DNA damage responses, antioxidant defense and metabolic changes. All these processes are relevant for the understanding of the pathogenesis of T2D, and also for the development of diabetic complications in chronic patients. Recently, common characteristics linking T2D to Alzheimer's disease (AD) have been reported. The purpose of this chapter is to highlight the main processes associated with the disease, such as insulin signaling pathways, oxidative stress, mitochondrial dysfunction, DNA damage and repair and antioxidant defense. In addition, the molecular impact of nutritional interventions in patients with T2D will also be addressed, as will the molecular keystones linking T2D and AD. Recently, there is accumulated evidence indicating that the two diseases may share common signaling pathways that may be relevant to the etiopathogenesis of each of them.

Keywords: type 2 diabetes mellitus, insulin resistance, hyperglycemia, oxidative stress, DNA repair, reactive oxygen species (ROS), mitochondrial dysfunction, Alzheimer's disease

1. Introduction

Diabetes mellitus is a metabolic disease that has a major impact on global public health, affecting more than 425 million people worldwide. The number of affected people tends to increase, mainly due to obesity, a risk factor closely related to type 2 diabetes mellitus (T2D), the most common form of diabetes. Hyperglycemia is the most striking feature of the disease, which is the increase of blood glucose levels above those presented by healthy individuals. This could be the main consequence of poor insulin secretion, lack of insulin sensitivity in target tissues or the combination of both [1, 2].

The genetic predisposition may be one of the determinants that favor the susceptibility to T2D development. Several variants of genes and even epigenetic

modifications in histones and DNA methylation may influence the heritability of T2D [3, 4]. Due to the complexity of the interaction of different factors involved in this disease, genome-wide association studies (GWAS) have been performed in an attempt to identify genetic variants related to the increased risk of T2D.

In 2007, the first GWAS was performed in France in patients with T2D [5]; At present, at least 75 associated loci have been identified, including the *TCF7L2* transcription factor, which is the most common gene found, in addition to *PPARG*, *KCNJ11*, *FTO*, *CDKN2A/2B*, *CDKAL1*, *IGFBP2* among others [6]. Since then, similar studies showed that the loci presenting greater association with T2D vary as regards the relative risk between different ethnicities [7]. Besides, these variants explain only a low percentage of the disease heritability, most of which are found in intergenic or intronic regions [6]. Furthermore, DNA methylation patterns may contribute to genetic susceptibility to T2D. There is evidence of an increased risk of T2D development associated with distinct methylation patterns in some loci [8], but this approach is still a major challenge for researchers.

While obesity and overweight have been considered an important cause of T2D, a poor diet and lack of physical activity significantly contribute to an increased risk of insulin resistance and T2D [9].

One of the greatest concerns regarding the poor glycemic control in patients with T2D is related to the micro and macrovascular complications of diabetes. Since the onset of T2D did not present specific acute symptoms, 50% of adults with T2D do not know that they have the disease [9]. Chronic hyperglycemia induces a series of complications, such as retinopathy, neuropathy and nephropathy. In a long term, the high blood glucose levels may also induce endothelial dysfunction, which contributes to the increased risk for the development of cardiovascular diseases.

2. Oxidative stress and mitochondrial dysfunction

Changes in glucose homeostasis represent a critical factor for the development of metabolic diseases. Normally, to maintain optimal levels of blood glucose, the pancreas secretes two hormones. In response to high glucose levels, pancreatic β cells secrete insulin, which promote the uptake of glucose by peripheral tissues, reduce gluconeogenesis and decrease glycogen and triglyceride breakdown. However, when glucose levels are reduced in the blood, α -cells release glucagon, which will reverse the above process.

Overall, insulin resistance is one of the main causes of disturbances in glucose homeostasis; when insulin receptors do not respond to the amount of insulin produced, the consequence is a deficiency of the body in the glucose uptake and absorption. As a compensatory mechanism, pancreatic β cells increase the release of insulin, but if the glucose levels remain high due to the inability of insulin to achieve body's demand, it may occur the onset of T2D. Insulin resistance persists in patients since pre-diabetes, a stage in which individuals show glucose levels above the normal values, but not so high for the diagnosis of the disease. It should be mention that at this stage, a healthy nutritional style, physical exercises and weight control may allow the individuals to recover normal glucose levels.

At a long term, high levels of blood glucose can lead to a number of cellular and molecular changes in the body, especially due to the production of reactive oxygen species (ROS) [10]. It is well known that mitochondria are the main source of ROS; these highly dynamic organelles constantly undergo structural changes, responding rapidly to the physiological alterations in the environment. Exposure of cells to hyperglycemic conditions is associated with several mitochondrial alterations. There is evidence that the number and morphology of mitochondria are essential

for the maintenance of cellular function. Hyperglycemia in this context is reported as an inducer of glucose metabolism, which can promote several conformational changes in mitochondria, overload of the electron transport chain, leading to the overproduction of ROS, and mitochondrial dysfunction [11–14].

It has been reported [15] that patients with pre-diabetes presented an increase in the mitochondrial mass, suggesting that the initial increase in blood glucose levels may induce an adaptative response in order to increase mitochondrial biogenesis to maintain homeostasis. These results are associated with an increase in mitophagy, raising evidence that during pre-diabetes state there may be an elimination of compromised mitochondria in an attempt to reduce mitochondrial oxidative stress [15, 16].

ROS are normal byproducts of aerobic respiration, consisting of non-radicals, as hydrogen peroxide (H_2O_2) and free radicals, as hydroxyl radical (OH) and superoxide anion (O_2^-). In normal situations, antioxidant enzymes (glutathione peroxidase, catalase and superoxide dismutase) are able to eliminate ROS and maintain the homeostasis of the organism. However, in a hyperglycemic state, the mitochondria electron transport chain becomes hyperactive, thus inducing an excessive production of ROS that surpasses the antioxidant defense system [17]. The imbalance between the prooxidants and the antioxidant defense system lead to a condition called oxidative stress, where the reactive molecules can cause damage to lipids, proteins and nucleic acids [18].

Among DNA damage caused by ROS, the major oxidized base modifications generated are 8-oxoguanine (8-oxoG) and 8-oxodesoxyguanosine (8-oxodG), which could occur in both DNA and the nucleotide pool, the latter can be incorporated into the DNA during replication or repair [19, 20]. The repair of 8-oxoG in DNA is performed by the base excision repair mechanism (BER), in which the DNA glycosylase OGG1 recognizes the 8oxoG and together with APE1 enzyme, polymerase complex β and DNA ligase I promote DNA repair [21, 22]; the removal of 8-oxo-dG from the nucleotide pool is performed by the enzyme hMTH1 (human MutT homolog), which hydrolyses 8-oxo-dGTP to transport this molecule to the cytosol, preventing its incorporation into the DNA [23]. For different types of DNA lesions, other DNA repair processes, such as nucleotide excision, homologous recombination, non-homologous end-joining, and mismatch repair may also occur. In diabetes, there is evidence that DNA repair levels and activity of antioxidant enzymes are reduced [24, 25], as well as DNA damage levels and oxidized bases in these patients were found increased [26, 27].

The oxidative stress promoted by chronic hyperglycemia causes cellular damage mainly in the pancreatic β cells, which present low levels of antioxidant enzymes, and are more susceptible to damages caused by ROS. This stress is also responsible for releasing inflammatory mediators, which in turn culminate in a vicious cycle leading to β -cell dysfunction, insulin resistance and metabolic decline, which are critical for the development of T2D [28].

In diabetes, high glucose levels may also induce endoplasmic reticulum (ER) stress. Since the ER is the main responsible for protein maturation and folding, in particular proinsulin, in a hyperglycemic state, this molecule tends to be excessively synthesized and can overload the ER, leading to the accumulation of misfolded proteins, thus generating a stress condition. This stress may lead to the activation of the unfolded protein response pathway, which may restore ER homeostasis or induce cell death. The latter may lead to β -cell dysfunction, and consequently, to the reduction of insulin secretion and chronic hyperglycemia [28–31].

Several metabolic pathways are involved in insulin resistance and induction of inflammation and stress, including the JNK (JUN N-terminal kinase) and IKK β (IkB kinase- β) pathways, both of them can be activated by ER stress [32]. IKK β is

a protein responsible for mediating the activation of NF- κ B (nuclear factor- κ B), which in turn stimulates the proinflammatory cytokines, TNF- α (tumor necrosis factor-alpha) and interleukin 1 β (IL-1 β), that can promote inhibition of the insulin receptor substrate (IRS) protein phosphorylation or reduce their transcriptional expression, compromising the insulin pathway and contributing to insulin resistance [25, 28].

Obesity is another critical factor that results in oxidative stress and insulin resistance [33], generating a chronic inflammatory condition in adipose tissue, causing the recurrent release of pro-inflammatory cytokines, such as those previously mentioned, in addition to interleukin 6 (IL-6), which together lead to pancreatic β -cell dysfunction, decreased insulin secretion, and consequently hyperglycemia and thus triggering T2D [28].

3. Insulin signaling pathway

The normal signalization of the insulin signaling pathway is vital and its dysregulation is implicated not only in T2D but also in diseases such as cancer, cardiovascular and neurodegenerative diseases. Changes in this signaling cascade as well as the consequences thereof, makes this pathway an important subject of study, considering its relevance in terms of age-related diseases.

Normally, the transport of glucose into the cells occurs through different intracellular signaling mechanisms performed in cascade, as shown in **Figure 1**. Firstly, insulin binds to its receptor, promoting tyrosine phosphorylation of IRS proteins, especially IRS-1 and 2. The tyrosine phosphorylation is critical for the

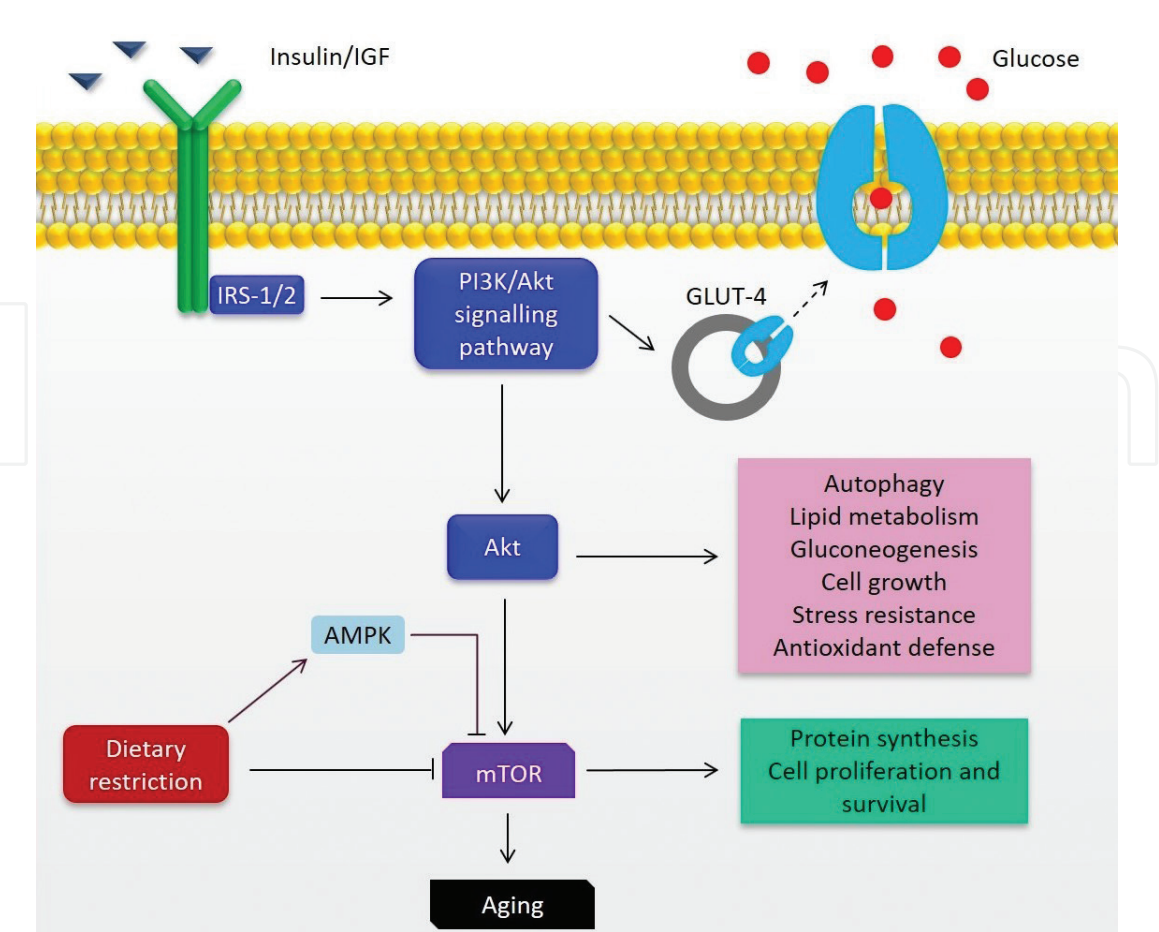


Figure 1.
Insulin/insulin-like growth factor signaling pathway.

correct activation of the insulin pathway. Phosphorylation at serine or threonine residues is associated with the inhibition or even degradation of IRS proteins promoting downregulation of the pathway. This inhibitory effect over the pathway occurs normally via insulin-induced kinases as a way to keep the correct function of all proteins involved. However, some conditions as hyperglycemia, release of proinflammatory cytokines, oxidative stress (due to mitochondrial dysfunction), in addition to elevated fatty acids and ER stress can induce an increased serine or threonine phosphorylation, promoting the downregulation of insulin signaling and exacerbating the insulin resistance condition [2, 34, 35].

Thus, tyrosine phosphorylation of IRS proteins, further activate PI3K (phosphatidylinositol 3-kinase) protein [36, 37], promoting in particular the translocation of glucose transporter 4 (GLUT4) to the plasma membrane enabling the entrance of glucose into the cell [38]. Among the PI3K-associated downstream proteins, here we focus especially on Akt (alpha serine/threonine-protein kinase) [39, 40]. Once activated, Akt-regulated proteins have a key role in metabolism, glycogen synthesis, autophagy, growth, cell survival, transcription and protein synthesis [41]. Akt has been described as an important downregulator of GSK3 α/β proteins. These proteins are strongly associated with the formation of amyloid beta and phosphorylation of tau protein, which are the main proteins involved in Alzheimer's disease [42, 43]. Another important target of Akt are the FOXO (Forkhead box O) transcription factors, which regulates the expression of different genes related to gluconeogenesis, lipid metabolism, resistance to stress, DNA repair, cell growth, survival, differentiation, among others [41, 44, 45]. The kinase mTOR (mammalian target of rapamycin), responsible for regulating cell growth and metabolism, being a large sensor of nutrients and cellular energy, is also a target of Akt [46–48] and has a major role in the mechanism of longevity extension [49].

There is evidence that changes in the expression of growth factors, IRS proteins, IGF-1, AKT, mTOR, FOXO among others that result in downregulation of the insulin signaling pathway through nutritional restriction, for example, are implicated in the resistance to stress, induction of autophagy, extension of longevity and reduction of aging-related diseases in different species, such as worms, flies, rats, mice and some primates [47, 50–55]. The inhibition of mTOR has been widely discussed as the main protein involved in the longevity extension. Metformin, a drug commonly used to control the glycemic levels in diabetics, is able to inhibit the activity of mTOR, via activation of AMPK, a protein with role in glycolysis, fatty acid oxidation, lipogenesis reduction, gluconeogenesis and protein synthesis [52, 56, 57]. AMPK is also important in mitochondrial biogenesis, since it activates PGC1 α [58], which has the ability to stimulate the mitochondrial electron transport chain and suppress ROS levels, being essential in inducing the antioxidant defense system [49, 59].

4. Impact of nutritional interventions in diabetes care

Diabetes is a global health problem. Currently, the treatment of this disease has been carried out with medications, such as metformin, aiming to reduce the blood glucose levels, in an attempt to prevent a series of alterations in the cellular metabolism caused by chronic hyperglycemia. However, the success of treatments, in general, is limited, requiring other types of interventions (nutritional and regular physical activity, mainly) related to the patients' lifestyle. The majority of patients with T2D present age between 40 and 59 years, which is critical for the disease [9] and in this phase, as in the subsequent stages, with the progression of the aging process, the protein homeostasis becomes increasingly compromised,

also accompanied by a reduction in the efficiency of the DNA repair system and the antioxidant defense, besides the organism as a whole, consequently leading to the accumulation of cellular damage [51, 58, 60, 61].

A great number of patients with T2D are overweight or obese. Changes in the lifestyle have been shown essential in controlling the levels of blood glucose. Additionally, it was reported that T2D patients submitted to a 7-day intervention to achieve adequate blood glucose levels led to a significant decrease in DNA damage levels [26]. In particular, some nutritional interventions, as well as caloric (CR) or protein restriction, have been shown to be very effective, not only for reducing blood glucose levels, but also for having very positive benefits in terms of increased life expectancy, as demonstrated in several model organisms [52, 54], in addition to reducing the incidence of aging-related diseases [62–64].

A major recruitment study known as CALERIE (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy) aimed to show the effects of caloric restriction in humans. It has already been shown that in a period of 2 years, the CR is very efficient in improving insulin sensitivity [65], reducing inflammatory markers [66] and reducing oxidative stress [67]. Those features are especially significantly increased in patients with T2D, which would make this approach a valuable intervention for treatment of those patients. In fact, in a study performed in rhesus monkeys, from the 38 control animals, 16 developed high levels of blood glucose, becoming either prediabetic or diabetic. On the other hand, all the animals under caloric restriction did not present any impairment on glucose regulation [64], which may demonstrate the importance of this kind of intervention.

Although this approach has been widely discussed, the studies are still controversial regarding the best diet composition for diabetic patients. It has been hypothesized that a high intake of proteins could influence the effects of a caloric restriction [68]. In fact, there is a study showing the efficiency of a protein restriction intervention on reducing cancer incidence and extending lifespan regardless the intake of calories [69].

5. Susceptibility of T2D to Alzheimer's disease

Alzheimer's disease (AD) is a progressive, continuous neurodegenerative disorder that affects large areas of the cerebral cortex and hippocampus. These abnormalities are usually detected for the first time in brain tissue involving the frontal and temporal lobes and then slowly advance to other areas of the neocortex at rates that vary considerably between individuals [70]. By 2018, an estimated 50 million people are living with dementia, with AD being the most prevalent form [71]. The main symptoms of AD result from the formation of beta-amyloid (A β) plaques and neurofibrillary tangles of the tau protein in the brain, which together lead to neuronal dysfunction and death, causing memory loss episodes which are characteristic of the pathology [70, 72]. It has been reported that similar to the toxicity caused by A β aggregates in the brains of patients with AD, amyloid deposits in the pancreas occur in patients with diabetes, which may induce the death of pancreatic insulin-producing β cells [73].

Recently, several studies have narrowed the relationship between T2D and dementias [74, 75], suggesting that in addition to an increase in the incidence of dementia in T2D patients, a more rapid cognitive decline may also occur, including a higher conversion rate of individuals who have mild cognitive impairment in patients with dementia [76–78]. This information has aroused interest in studying a possible association between T2D and dementia.

Many hypotheses have been raised about the common features that involve the two pathologies and how these can be related to each other. It has been suggested that both diseases may share common signaling pathways, although molecular and cellular mechanisms still need elucidation. There is evidence that insulin resistance in the brain, including insulin pathway dysregulation, inflammatory processes, formation of advanced glycation products, as well as oxidative stress and mitochondrial dysfunction, may be implicated in the pathogenesis of AD and T2D [79].

Insulin, besides having an essential role as regulator of energy metabolism, also exerts a role in plasticity, survival and neuronal growth, as well as learning and memory processes, contributing to the improvement of cognitive functions; their absence has been associated with cognitive decline in patients with neurological and neurodegenerative diseases, such as AD [80–82]. In fact, it has been demonstrated that brains of patients with AD present altered insulin signaling [83]. Insulin receptors are found in the central nervous system (CNS) in large number and their impairment (hence the signaling cascade) may culminate in a number of alterations mainly involving PI3K, AKT and mTOR proteins. Abnormal expression of these and other proteins and the deregulation of this pathway may contribute to the formation of A β aggregates, neurofibrillary tangles by hyperphosphorylation of the tau protein [84], as well as the impairment of the autophagy process regulated by mTOR [75], whose hyperexpression has been related to T2D [85] and AD [86].

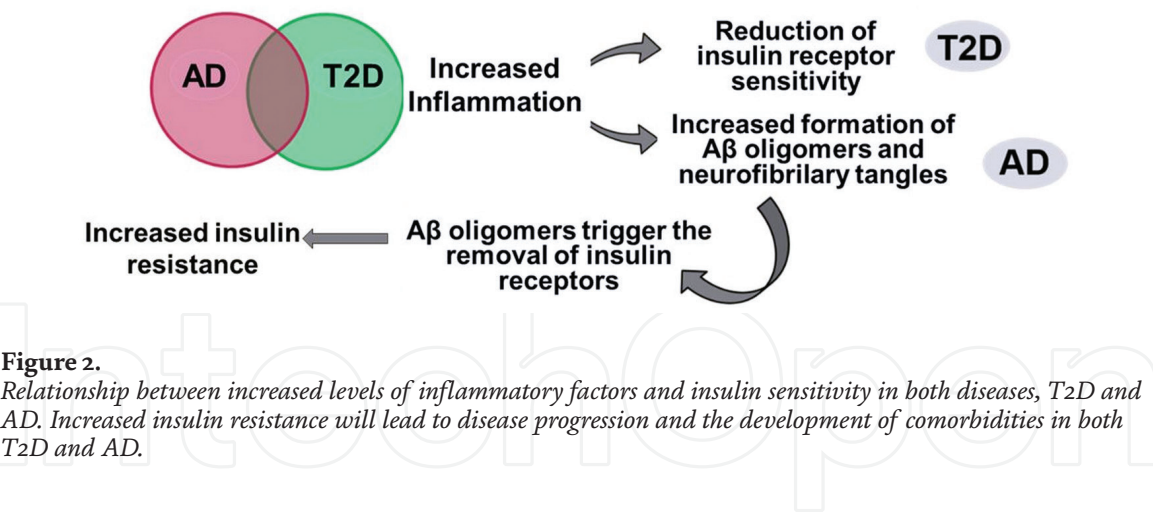
Thus, in the same way as T2D, AD is a disease related to less efficient molecular signaling in response to insulin, inflammation, oxidative stress, formation of advanced glycation end products and increased accumulation of DNA damage [87–89]. Thus, these characteristics suggest a connection between the two diseases.

The presence of higher levels of inflammation has already been described both in T2D and AD patients. In T2D there is a chronic inflammatory response localized in adipose tissue and characterized by the infiltration of immune system components, mainly macrophages, which release different proinflammatory cytokines such as TNF- α and IL-6 [90, 91]. Such cytokines may lead to insulin resistance by inducing cytokine signaling suppressors (SOCS), which participate in the degradation of IRS-1 and IRS-2 [92, 93]. In addition, these cytokines also activate stress response kinases, such as JNK and NF- κ B, which in turn act on the insulin receptor, inhibiting its tyrosine kinase activity, therefore culminating in insulin resistance [94, 95].

Similar inflammatory processes probably occur in the brain and peripheral tissues. Several studies have established the presence of inflammatory markers in the brains of patients with AD, including high levels of cytokines/chemokines [96]. In addition, inflammatory mediator levels in blood as TNF- α , IL-6 and IL-1b are increased in AD patients [97]. Thus, both in the brain and in peripheral tissues, chronic inflammation becomes harmful, leading to progressive damage to tissues and consequently triggering degenerative diseases.

There is evidence that insulin plays an important role in glucose regulation in the CNS, and its additional effects on neurons include metabolic, neurotrophic, neuromodulatory and neuroendocrine actions [98]. The presence of higher levels of inflammatory mediators in the CNS seems to stimulate the formation of beta-amyloid oligomers and neurofibrillary tangles, which trigger the removal of insulin receptors in neurons, making this condition common in both T2D and AD, triggering progression of both diseases [89, 99] (**Figure 2**).

Besides, the lower sensitivity to insulin, in addition to being important for the progression of T2D, also appears to affect the expression and metabolism of A β proteins in the CNS, and consequently, an increase in oxidative stress condition [2, 100], which in turn, induces greater accumulation of A β oligomers [101] and the release of inflammatory mediators [88], as already mentioned. Thus, it seems



that all these processes are related to T2D and AD as a vicious cycle, leading to the development and progression of comorbidities in both diseases, being one of the consequences of hyperglycemia and the accumulation of A β oligomers, respectively.

However, both diseases seem to present less efficient DNA repair processes, which generate genomic instability and also cell death; this condition is closely related to the complications reported for patients with T2D, and also AD [24, 102]. According to Xavier et al. [103], hyperglycemic T2D patients presented induction of DNA repair pathways, probably in response to higher levels of oxidative stress, but it remains to be elucidated whether the efficacy of repair pathways are normal in non-hyperglycemic T2D patients. In the case of AD, there is evidence that repair of DNA double strand breaks is less efficient [104], as well as base excision repair pathway [105], which would be detrimental to AD individuals, considering the relevance of DNA repair mechanisms for the DNA damage repair caused by ROS [106], and also by several kinds of endogenous and exogenous agents.

6. Conclusions

Insulin resistance is one of the main causes of disturbances in glucose homeostasis. In patients with T2D, long term exposure to high levels of blood glucose can lead to a number of cellular and molecular changes in the body. In this context, hyperglycemia can promote several conformational changes in mitochondria, overload of the electron transport chain, leading to the overproduction of ROS, and mitochondrial dysfunction. Furthermore, the imbalance between the prooxidant and the antioxidant defense system lead to a condition of oxidative stress, where the reactive molecules can cause damage to lipids, proteins and nucleic acids. Interestingly, there is evidence that DNA repair levels and activity of antioxidant enzymes are reduced in T2D; in the opposite, DNA damage levels as well as oxidized bases in these patients were found increased. Insulin resistance has been also associated with several metabolic pathways and induction of inflammation and stress, including ER stress. Therefore, the normal signalization of the insulin pathway is vital and its dysregulation is implicated not only in T2D but also in other diseases such as cancer, cardiovascular and neurodegenerative diseases. In the brain, there is also evidence of insulin resistance and dysregulation of insulin pathway, generating inflammatory processes, as well as oxidative stress and mitochondrial dysfunction, all of them might be implicated in the pathogenesis of T2D and AD, thus linking the two diseases.

Acknowledgements

Research supported by Coordination for the Improvement of Higher Education Personnel (CAPES), National Council for Scientific and Technological Development (CNPq) and São Paulo Research Foundation (FAPESP). We thank CNPq for providing fellowship to J.E.B.F.Lima.

Conflict of interest

There is no conflict of interests.

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