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Role of dietary and endogenous antioxidants in diabetes

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1. Abstract

Diabetes affects different people of all ages, race and sex. This is a condition characterized by a state of chronic hyperglicaemia that lead to an increase of intracellular oxidative stress linked to the overproduction of free radicals. In the present review we focus our attention on the molecular mechanisms leading to oxidative stress- mediates complications with particular regard to central nervous system (CNS). Furthermore the present review reports the effects of different kind of antioxidants with enzymatic and non enzymatic action that may significantly decrease the intracellular free radicals overproduction and prevents the hyperglicaemia-mediated complications.

Key Words: Antioxidants, Diabetes, central nervous system, diet

2. Introduction

Diabetes affects different people of all ages, race and sex. It is estimated that worldwide there are approximately 150 million people with diabetes mellitus 30 million of which living in Europe, and more than 3 million in Italy (Weekers and Krzesinski, 2005). This is a condition characterized by a relative or absolute insufficiency of insulin secretion and the concomitant

insensitivity or resistance to the metabolic action of this hormone in its target tissues. It follows a state of chronic hyperglycaemia (Ali and El-Remessy, 2009). The acute and chronic complications related to this of disorder are several. Acute complications are related to the almost total lack of insulin. In these cases the patient may have a ketoacidosic coma due to accumulation of abnormal products of metabolism, the ketones, which cause loss of consciousness, dehydration and severe cellular homeostasis alterations. Chronic complications involve different organs and tissues, including eyes, kidneys, heart, blood vessels and peripheral nerves. (Table 1)

Cells that are particularly affected by hyperglycemia are those who are unable to reduce the transport of glucose into the cell (Heilig *et al.*, 1995, Kaiser *et al.*, 1993). Thus, diabetes selectively damages endothelial and mesangial cells, leading to a high concentration of glucose within the cell. In the present review we will focus our attention on the molecular mechanisms leading to oxidative stress- mediated complications with particular regard to central nervous system (CNS). It is well known that diabetes affects all cells of all tissues.

2.1. Polyol flux

This pathway is related to mechanisms operating intracellularly rather than in the extracellular space(Gabbay *et al.*, 1966)(Figure 1). The first enzyme of the pathway the Aldose-reductase, normally reduces toxic aldehydes generated by reactive oxygen species (ROS) intracellularly, to inactive alcohols, but when the intracellular concentration of glucose becomes too high, the Aldose-reductase reduces the surplus of glucose to sorbitol, which is subsequently metabolized to fructose. In the process of reduction of intracellular glucose to sorbitol, the Aldose reductase

consumes the cofactor NADPH as a cofactor while Sorbitol dehydrogenase (SDH) oxidizes sorbitol to fructose using NAD^+ (Brownlee, 2001, Lee and Chung, 1999).

However as shown in Figure 1, NADPH is also the essential cofactor for the regeneration of an important intracellular antioxidant: reduced glutathione (GSH). Consistently decreased levels of GSH has been found in the lenses of transgenic mice that overexpress aldose reductase, and this is the most likely mechanism by which increased flux through the polyol pathway has deleterious consequences such as increases susceptibility to intracellular oxidative stress (Lee and Chung, 1999). This conclusion is further supported by recent experiments with homozygous knockout mice deficient in aldose reductase, showing that, diabetes neither decreased the GSH content of sciatic nerve nor reduced motor nerve conduction velocity (Brownlee, 2001).

2.2. Advanced Glicosylation Products (AGE)

Another important mechanism playng a major role in the pathophysiology of complications related to hyperglycemia is the production of advanced glycosylation products (AGE) that appear to damage cells through at least three distinct mechanisms (Brownlee, 2005). The first mechanism is the modification of intracellular proteins in endothelial cells, including proteins involved in the regulation of gene transcription. The second mechanism concerns the ability of these AGE precursors to diffuse out of the cell and modify the molecules of the surrounding extracellular matrix (McLellan *et al.*, 1994), thereby altering the crosstalk between the matrix and the cell causing cellular dysfunction (Charonis *et al.*, 1990). Finally, the third mechanism, regard the spread of these precursors outside the cell and their complexes with serum albumins (Brownlee, 2001) (Figure 2).

These circulating modified proteins interfere with the normal remodelling process of vessels, leading to abnormalities of cell turnover and extracellular matrix, vascular homeostasis, vascular permeability and coagulation all leading to vascular disease (Li *et al.*, 1996, Vlassara *et al.*, 1988). To this regards Hans-Peter Hammes et al. (Hammes *et al.*, 1991), showed that pharmacological inhibition of AGE precursors prevents late structural changes of experimental diabetic retinopathy.

Vascular complications primarily affect those not requiring insulin for glucose uptake, such as blood vessels, kidneys and CNS (Meier *et al.*, 2009).

2.3. Protein Kinase C pathway

Hyperglycaemia is the main stimulus that leads to PKC activation because increases the synthesis of diacylglycerol but also include non-esterified fatty acids (NEFAs), and various growth factors, including angiotensin II (Hua *et al.*, 2003, Inoguchi *et al.*, 2003). PKC activation is involved in the regulation of vascular permeability and contractility, endothelial cell activation and vasoconstriction, extracellular matrix (ECM) synthesis and turnover, abnormal angiogenesis, excessive apoptosis, leucocyte adhesion, abnormal growth factor signalling and cytokine action, as well as abnormal cell growth and angiogenesis, all of which are involved in the pathophysiology of diabetic vascular complications (Idris *et al.*, 2001, Inoguchi *et al.*, 2003, Meier and King, 2000) (Figure 3).

Activation of PKC leads also to the production of phosphatidylserine, phorbol ester, vascular endothelial growth factor (VEGF), activation of nuclear factor κB (NFκB) (Brownlee, 2001, Gould and Newton, 2008, Parker and Murray-Rust, 2004).

2.4. Hexosamine pathway

Another event that might also cause several manifestations of diabetic complications is an increased flux through the hexosamine pathway (Brownlee, 2005). As shown schematically in Figure 4, when intracellular concentration of glucose is high, much of this glucose is metabolized through the process of glycolysis. However, some of the fructose 6-phosphate is diverted into a pathway of signaling where an enzyme called glutamine: fructose-6 phosphate amidotransferase (GFAT) converts fructose 6-phosphate into glucosamine-6 phosphate and finally to UDP-N-acetyl glucosamine. Subsequently the N-acetyl glucosamine (GlcNAc) is placed in contact with residues of serine and threonine of transcription factors causing pathological changes in gene expression (Brownlee, 2005, Kolm-Litty *et al.*, 1998, Wells and Hart, 2003).To this regard, it was shown that in Figure 4, the increased glycosylation of Sp1 transcription factor induces the expression of inibitor-1 activator of plasminogen (PAI-1), which is released in the process of fibrinolysis playing a major role in blood vessels of diabetics (Du *et al.*, 2000) (Figure 4). Therefore, modification of Sp1 by GlcNAc may regulate other glucose-responsive genes in addition to that for PAI-1 (Figure 4).

As virtually every RNA polymerase II transcription factor examined has been found to be O-acetylglucosaminylated (Hart, 1997). It is possible that reciprocal modification by O-acetylglucosaminylation and phosphorylation of transcription factors other than Sp1 may function as a more generalized mechanism for regulating glucose-responsive gene transcription. Thus, activation of the hexosamine pathway by hyperglycaemia may result in many changes in both gene expression and protein function, which together contribute to the pathogenesis of diabetic complications (Brownlee, 2005).

3. How hyperglycaemia induce increased oxidative stress.

Brownlee et al. (Brownlee, 2001) showed that the process for unifying all the above pathogenic mechanisms consist in the increased production of ROS in mitochondria. These findings are consistent with the four-pathway involved in the development of diabetic complications (increased polyol flux, increased formation of advanced AGE, activation of PKC and increased flux through the hexosamine pathway) and with the unifying hypothesis of the effects of hyperglycemia in endothelial dysfunction (Du *et al.*, 2000, Nishikawa *et al.*, 2000). Brownlee et al. (Brownlee, 2001, Rosca *et al.*, 2005) have determined that the source of free radicals in endothelial cells incubated with high glucose recides in the transport of pyruvate derived from glycolysis, in the mitochondria, at the level of complex II (succinate ubiquinone oxidoreductase), one of the four complexes associated to inner membrane with central role in oxidative phosphorylation. Specifically in the mitochondrial transport chain of electrons there are four protein complexes, called complex I, II, III and IV (Figure 5).

Since the electrons are transported from complex I to molecular O_2, some energy of those electrons is used to pump protons across the membrane at complex I, III, and IV. This generates what is effectively a voltage difference across the mitochondrial membrane. The energy resulting from this gradient leads to the synthesis of ATP through the ATP synthase (Trumpower, 1990, Wallace, 1992). Alternatively, non-coupled proteins (UCPs) can spread along the voltage gradient to generate heat to keep the rate of ATP generation constant. In contrast, in presence of high glucose concentration, there is more substrate to be oxidized in the TCA cycle, which, in fact, pushes more electron donors (NADH and $FADH_2$) in the transport chain electrons (Figure 5).

Consequently, the voltage gradient across the mitochondrial membrane increases until a threshold limit. Therefore, the transfer of electrons within the complex III is blocked (Korshunov *et al.*, 1997), leading to the return of electrons to coenzyme Q, which give one by one the electrons to molecular oxygen, thereby generating superoxide (Figure 5).

Although ROS have a physiological role in signal transduction, it is convincible that their prolonged and sustained generation in endothelial cells exposed to high glucose may have considerable effects on the properties of the cell. The importance of this observation is that the present mechanism is able to activate all the others involved in the pathogenesis of diabetic complications (Brownlee, 2001, Du *et al.*, 2001). Many other pathways, however, can be considered as candidates for the formation of free radicals of oxygen during hyperglycemia. These pathways include: NAD(P)H oxidase, xanthine oxidase, arachidonic acid cascade (lipo oxygenase and cycle oxygenase), and microsomal enzymes (Cross and Jones, 1991). Furthermore, activation of NF-kB through these mechanisms links hyperglycemia to the expression of multiple genes related to vascular stress response (Collins, 1993).

Furthermore increased superoxide production during hyperglycemia is a key event of the activation of other pathways involved in the pathogenesis of diabetic complications but it represents only the first step in the production of endothelial dysfunction in diabetes.

The production of nitric oxide (NO) plays a central role in the modulation of endothelial function (Moncada *et al.*, 1991). NO is generated from the metabolism of L-arginine mediated from enzyme nitric oxide synthase (NOS), of which there exists three isoforms: two constitutive forms, cerebral (bNOS) and endothelial (eNOS) and an inducible (iNOS) (Nathan and Xie, 1994). iNOS is induced by various stimuli, including hyperglycemia (Baek *et al.*, 1993), while

superoxide generated by mitochondria may inhibit the constitutive enzyme eNOS, although sufficient NO is still produced (Du *et al.*, 2001). Superoxide anion can neutralize NO, thus reducing the effectiveness of vasodilatation and the evidence suggests that during hyperglycemia (Benz *et al.*, 2002), there is a reduced availability of NO (Hink *et al.*, 2001). Hyperglycaemia also increases, by NF-kB activation, the expression of both NAD(P)H oxidase and iNOS (Spitaler and Graier, 2002). The increase of superoxide, when combined with the increase of NO, promotes the formation of peroxynitrite a strong oxidant, which in turn oxidizes the tetrahydrobiopterina to dihydrobiopterin (Beckman and Koppenol, 1996), a cofactor of iNOS (Guzik *et al.*, 2002). In terms of lack of tetrahydrobiopterin, iNOS appears uncoupled and this involves the deviation of the flow of electrons from L-arginine to molecular oxygen, resulting an increased production of superoxide compared to NO (Brodsky *et al.*, 2002, Cosentino *et al.*, 1997). Exposure to peroxynitrite during hyperglycemia produces also a decoupling of eNOS, presumably due to depletion of zinc of the enzyme, thus promoting the overproduction of superoxide (Zou *et al.*, 2002). The anion peroxynitrite results cytotoxic because it can oxidize sulphydryl groups of proteins, to initiate lipid oxidation and the nitration of the amino acids, such as tyrosine, which affect many signal transduction pathways (Beckman and Koppenol, 1996) (Figure 6).

Furthermore, peroxynitrite is a potent initiator of DNA damage to a single helix. This alteration of the DNA is the necessary stimulus for activation of poly (ADP-ribose) polymerase (PARP), a nuclear enzyme that repairs damage to DNA (Garcia *et al.*, 2001). As described above, when endothelial cells are exposed to high levels of glucose, they produce reactive nitrogen and ROS (Garcia *et al.*, 2001). These reactive species damage the DNA single helix thus activating the

enzyme PARP (Garcia *et al.*, 2001). The role of hyperglycaemia and related oxidative stress in producing DNA damage is supported by the detection of increased levels of 8-hydroxyguanine and 8-hydroxydeoxyguanosine (8-OHdG) (a marker of oxidative damage to DNA) in plasma and tissues of diabetic rats because this deoxyguanosine (dG) one of the bases of the DNA, once oxidized turns into 8-OHdG, and through techniques immunostaining were unable to detect the presence (Liao *et al.*, 2005, Park *et al.*, 2001). These concentrations were related to the glucose level and were reduced by controlling hyperglycaemia itself or through the use of antioxidants such as probucol and vitamin E (Park *et al.*, 2001). The enzyme PARP, when activated, catalyzes the reaction of attack of the ADP-ribose from NAD^+ to nuclear proteins, thus splitting the NAD^+ in its two components: ADP-ribose and the nicotinamide mononucleotide. To replace the consumption of NAD^+, due to the activation of PARP, ATP is consumed. If the consumption is high, the cell may dye following the depletion of the energy substrate (Eliasson *et al.*, 1997). PARP activity also slows down glycolytic flux, the electron transport and the formation of ATP and produces ADP ribosilation of GADPH, a multifunctional enzyme (Kamoshima *et al.*, 1997). A recent study demonstrated the key role of GADPH such as ADP ribosilation leading to the activation of three major pathways of hyperglycaemic damage: PKC activation, the increased flux through the hexosamine pathway, and the formation of AGE (Du *et al.*, 2001). Inhibition of GADPH was also able to activate the transcription of pro-inflammatory factor NF-kB (Brownlee, 2001, Eliasson *et al.*, 1997) (Figure 7). The addition of an inhibitor of PARP has been able to block completely the activation of these pathways by hyperglycaemia (Kamoshima *et al.*, 1997). PARP, in addition to its catalytic activity, may act either as a co-activator, or as repressor of other transcription factors (Ha *et al.*, 2002).

4. Diabetes and CNS

In recent years, significantly more interest has been dedicated to the effect of diabetes on the brain**.** Many studies have found that the diabetes has a negative impact on cognition (Brands *et al.*, 2005). Diabetes has been associated with cognitive deficits, including psychomotor efficiency, learning and memory, cognitive functions, and executive functioning and also on control of sleep-wake states (Brands *et al.*, 2005, Strachan *et al.*, 1997). Prospective studies have found that women with diabetes had lower baseline cognitive scores than those without diabetes, and over time, they experienced an accelerated cognitive decline even after accounting for confounding factors such as hypertension, depression, stroke, smoking, and physical activity. There appears to be an association between severity of diabetes and the degree of brain involvement.

In addition, hyperglycaemia is unlikely to be the only factor in the development of cognitive impairments in type 2 diabetes: previous studies in patients with diabetes do not invariably show an association between chronic hyperglycaemia, as assessed by HbA1 levels, and the severity of cognitive impairments (Stewart and Liolitsa, 1999, Strachan *et al.*, 1997). Moreover, changes in cognition may already develop in 'pre-diabetic stages', such as impaired glucose tolerance, or in newly. Type 2 diabetes and insulin resistance are closely associated with factors such as obesity, atherogenic dyslipidaemia [elevated triacylglycerol level, small LDL (low-density lipoprotein) particles, low HDL (high-density lipoprotein) cholesterol], raised blood pressure, and pro-thrombotic and pro-inflammatory states. Together these factors constitute the metabolic syndrome, or insulin resistance syndrome (Meigs, 2000). A number of factors from this syndrome have been identified as independent predictors of cerebrovascular disease,

ischaemic stroke and accelerated cognitive decline and dementia e.g. (Kalmijn *et al.*, 1995, Kalmijn *et al.*, 2000, Kuusisto *et al.*, 1993, Kuusisto *et al.*, 1997). The combined occurrence of these risk factors in the metabolic syndrome might reinforce these effects (Golden *et al.*, 2002, Kernan *et al.*, 2002, Kuusisto *et al.*, 1993, Yaffe *et al.*, 2004). Given the clustering of insulin resistance, hypertension and dyslipidaemia in type 2 diabetes it may be difficult to determine which factor is the prime determinant in the development of cognitive dysfunction. The main question, however, is to determine if the metabolic syndrome is indeed a strong predictor of cognitive dysfunction in type 2 diabetes, and if this effect is (partially) independent of disturbances in glucose and insulin metabolism.

There is an association between diabetes and hippocampal atrophy (Stranahan *et al.*, 2008). Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons (Stranahan *et al.*, 2008). Furthermore, diabetes influences the hypothalamic-pituitary-adrenal axis, however the role of this neuroendocrine system in diabetes-induced cognitive dysfunction remains unexplored. It was demonstrated that, in both insulin-deficient rats and insulin-resistant mice, diabetes impairs hippocampus-dependent memory, synaptic plasticity and adult neurogenesis (Stranahan *et al.*, 2008). Rats treated with streptozocin have reduced insulin levels and show hyperglycemia, increased corticosterone, and impairments in hippocampal neurogenesis, synaptic plasticity and learning (Stranahan *et al.*, 2008).

Similar deficits was observed in db/db mice, which was characterized by insulin resistance, elevated corticosterone and obesity. Changes in hippocampal plasticity and function in both models are reversed when normal physiological levels of corticosterone are maintained, suggesting that cognitive impairment in diabetes may result from glucocorticoid-mediated

deficits in neurogenesis and synaptic plasticity (Stranahan *et al.*, 2008). The hippocampus, a limbic structure linked to higher brain functions, appears vulnerable in this diabetic subjects that have a higher risk of stroke, dementia, and cognitive decline. The dentate gyrus (DG) of the hippocampus is one of the limited neurogenic brain areas during adulthood; neurons born in the DG are involved in some types of learning and memory processes. Different study showed a decrease in the ability for proliferation and neuronal differentiation of newborn cells, measured by bromodeoxyuridine incorporation in the DG, from streptozotocin-induced diabetic mice. The hilar region, formed by mature neurons presenting higher sensitivity to brain damage, showed a reduced neuronal density in diabetic mice with respect to vehicle-treated mice. The antidepressant fluoxetine administered over a period of 10 days to diabetic mice was effective in preventing changes in proliferation and differentiation of new neurons (Beauquis *et al.*, 2009). Confocal microscope studies, including the use of neuronal and glial markers, suggested that differentiation toward a neuronal phenotype was decreased in diabetic animals and was reversed by the antidepressant treatment. In addition, the loss of hilar neurons was avoided by fluoxetine treatment. These features are common in some types of depression, diabetes, and aging processes, suggesting that they participate in the reported hippocampal abnormalities present in these conditions (Beauquis *et al.*, 2009).

Consistently with these observations it was demonstrated that Alzheimer increases amount of evidence links insulin itself to cognitive decline and dementia in type 2 diabetes (de la Monte and Wands, 2005, Gasparini and Xu, 2003, Watson and Craft, 2004). First, alterations in cerebral insulin receptor signaling may be involved, as a cerebral equivalent of peripheral insulin resistance. Secondly, insulin may affect the metabolism of Aβ (β- amyloid) and tau, two proteins

that represent the building blocks of amyloid plaques and neurofibrillary tangles, the neuropathological hallmarks of Alzheimer's disease and brain aging (de la Monte and Wands, 2005, Gasparini and Xu, 2003, Watson and Craft, 2004). Aging is associated with reductions in the level of insulin and the number of its receptors in the brain (Frolich *et al.*, 1998). In Alzheimer's disease this age-related reduction in cerebral insulin levels appears to be accompanied by disturbances of the insulin receptor signalling (Frolich *et al.*, 1998), leading to the qualification of Alzheimer's disease as 'an insulin-resistant brain state' (Hoyer, 1998). The relation between insulin and the metabolism of Aβ and tau is also receiving increasing attention (de la Monte and Wands, 2005, Watson and Craft, 2004). Aβ is derived from the so-called amyloid precursor protein. After secretion into the extracellular space Aβ can aggregate with other proteins, to form senile plaques. Alternatively, excessive Aβ can be cleared through LDL-receptor-related protein mediated endocytosis, or through direct extracellular proteolytic degradation (Ling *et al.*, 2003). This latter process involves IDE (insulin-degrading enzyme) (Farris *et al.*, 2003). Insulin appears to stimulate Aβ secretion, and inhibits the extracellular degradation of Aβ by competition from IDE (Gasparini and Xu, 2003).

A recent histophatological study of the hippocampus in pacient withalzheimer's disease reported marked reductions in IDE expression, and IDE mRNA levels, relative to controls (Cook *et al.*, 2003). Interestingly, this reduced expression only occurred in patients with the APOE (apolipoprotein E) ε4 allele. An interaction with the APOE ε4 genotype has also been demonstrated for the risk of Alzheimer's disease in diabetics patients, an observation that was supported by neuropathological results. Although the concepts of 'cerebral insulin resistance', and 'insulin-induced amyloid pathology' are an attractive explanation for some of the effects of

diabetes on the brain, there are still many loose ends. In contrast with the increasing body of knowledge on the mechanisms of insulin resistance in peripheral tissues (Le and Zick, 2001),very little is known about diabetes and how its treatment affect cerebral insulin and its receptor. Moreover, the knowledge on the role of insulin in brain physiology is far from complete. In particular, in apparent contrast with previous observations that hippocampal insulin receptor expression and signalling in rats are up-regulated following a spatial learning task in a water maze (Zhao *et al.*, 1999), mice with a targeted knockout of insulin receptors in the brain readily learn this same task (Schubert *et al.*, 2004). Hopefully, ongoing research in this rapidly evolving field of research will clarify these issues.

Another complication of diabetes is cerebral ischemia, or stroke. Diabetic patients are at an increased risk for stroke, with increased mortality and morbidity after a stroke (Bonow and Gheorghiade, 2004, Schubert *et al.*, 2004). Experimental animal models of stroke have confirmed that the extent of tissue damage is significantly worse in diabetic animals, although the mechanistic basis for this remains unknown.

Another possible cause of CNS dysfunction in diabetic patients is altered blood–brain barrier (BBB) function. The structural changes in cerebral microvessels, notably decreased capillary density in the cortex and basement membrane thickening (Affolter *et al.*, 1986, Jakobsen *et al.*, 1987, Mayhan, 1993, McCuskey and McCuskey, 1984), are important causes of diabetes-related changes in BBB function. In addition, cerebral ischemic episodes or coexisting hypertension and hyperosmolality will further contribute to diabetes-related deterioration of BBB (Arieff and Kleeman, 2000, Hatzinikolaou *et al.*, 1981, Klatzo, 1983, Mueller and Luft, 1982, Sage *et al.*, 1984). However,these factors cannot account for the loss of selective transport

properties of the BBB, and therefore other mechanisms have to be entertained. A partial list of potential mechanisms is shown in Table 2.

5. Therapeutic potential of dietary antioxidants

A potential therapeutic rationale for treatment of diabetic complications is based on the use of antioxidants to decrease oxidative stress. Antioxidants may eliminate free radicals toxic effects, as confirmed by evidence that people consuming fruits and vegetables rich in polyphenols and anthocyanins have a lower risk of serious complications following hyperglycaemia (Sies, 1997, Stanner *et al.*, 2004). Antioxidants restore the chemical balance in free radicals due to their ability to provide electrons which they are lacking. The human body naturally defends itself by free radicals producing endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHpx), glutathione reductase (GSSGR) and heme oxygenase (HO) (Stanner *et al.*, 2004). Between the classes of major antioxidants we can to mention : Plant Pigments (polyphenols and bioflavonoids), Vitamins (Vitamin C, Vitamin E and beta-carotene), Micro-nutrients and enzymes (selenium, copper, zinc, glutathione, coenzyme Q10, melatonin, uric acid, etc).

It should be noted that each antioxidant has a range limited to one or two specific free radicals. Thus, only complete and balanced diet can ensure an effective antioxydative action. To ensure an adequate daily intake of antioxidants, experts recommend a balanced diet and a daily consumption of fresh fruit and vegetables in season (Figure 8).

6. Antioxidant non-enzymatic action

5.1. Polyphenols

Made up of more rings of carbon atoms, these are pigments (natural coloring) present in nature. This group includes quercetin, flavonoids (yellow colored), anthocyanin (red-blue colored), etc. Polyphenols have anti-inflammatory, antiviral, antioxidant and antiallergic properties. They protect particularly from ischemic heart disease (i.e. coronary disease, stroke) and cancer (Barbosa *et al.*, 2008). However, there are many hundreds of polyphenols in the human diet, and it is not yet known if some compounds offer more protection than others (Loke *et al.*, 2010).

6.1.1. Resveratrol

It is a polyphenolic phytoalexin produced in appreciable amounts as a secondary metabolite in grapevines in response to fungal infections and shows the ability to reduce the risk of cardiovascular diseases (Fan *et al.*, 2008). It has been shown that the resveratrol has beneficial effects on the initiation and progression of atherosclerosis, including regulation of vasodilator and vasoconstrictor production, inhibition of oxidative stress/ROS generation, anti-inflammation, inhibition of modification of low-density lipoproteins and anti-platelet aggregation (Fan *et al.*, 2008). Furthermore, previous studies reported that dietary supplementation with resveratrol significantly reduced plaque formation in animal brains, and other neurodegenerative diseases (Karuppagounder *et al.*, 2009). In mice, oral resveratrol produced large reductions in brain plaque in the hypothalamus (-90%), striatum (-89%), and medial cortex (-48%) sections of the brain. In humans it is theorized that oral doses of resveratrol may reduce beta amyloid plaque

associated with aging changes in the brain. Researchers theorize that one mechanism for plaque eradication is the ability of resveratrol to chelate Cu^{++} (Karuppagounder *et al.*, 2009).

Based on the present knowledge, it appears to be a promising bioactive natural molecule with potential applications in phytotherapy or pharmacology. Furthermore the diabetic rats orally treated with resveratrol (5 mg kg^{-1} b.w d^{-1}) for 30 days resulted in a significant decrease in the levels of blood glucose, glycosylated hemoglobin, blood urea, serum uric acid, serum creatinine and diminished activities of pathophysiological enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) (Palsamy and Subramanian, 2008). The antihyperglycemic nature of resveratrol is also evidenced from the improvement in the levels of plasma insulin and hemoglobin. Current findings suggest that resveratrol may be considered as an effective therapeutic agent for diabetes mellitus treatment (Palsamy and Subramanian, 2008).

6.1.2. Cyanidins

They are largely distributed in the human diet through red-blue colored fruits, vegetables, grains, potatos, beans, and red wines, suggesting that we daily ingest significant amounts of these compounds from plant-based diets (Galvano *et al.*, 2007). Guo et al. extracted this anthocyanin from black rice, containing significant amounts of cyanidin 3-glucoside, and tested its protective effect on insulin sensitivity in cultured adypocites, which were exposed to hydrogen peroxide or tumor necrosis factor alpha (TNF-α). Dysfunction of adipocytes is strongly associated with the development of obesity and insulin resistance. The regulation of the expression of kinases by adypocites cells is an important target for the prevention of obesity and improvement of insulin sensitivity. It was observed that cyanidin 3-glucoside attenuated

hydrogen peroxide or TNF-α induced insulin resistance in a dose-dependent manner (Guo *et al.*, 2008). Pretreatment adipocytes cells with cyanidin 3-glucoside reduced the intracellular production of ROS and the activation of Jun N-terminal kinases. Cyanidin 3-glucoside also improves insulin sensitivity by regulating the glucose transporter 4 (GLUT-4) and retinol binding protein 4 system (Sasaki *et al.*, 2007). Cyanidin 3-glucoside reduces blood glucose level and improves insulin sensitivity due to the reduction of retinol binding protein 4 expression in diabetic mice. Finally, our recent study showed a protective effects of cyanidin 3-glucoside in rat brains feeding by ochratoxin A (data unpublished).

Scazzocchio et al. recently demonstrated that cyanidin-3-glucoside and its main intestinal metabolite protocatechuic acid increased adipocyte glucose uptake and GLUT4 membrane translocation. Significant increases in nuclear PPARγ activity, as well as in adiponectin and GLUT4 expressions were also shown. It is interesting that PPARγ inhibition counteracted the polyphenol-induced adiponectin and GLUT4 upregulations, suggesting a direct involvement of PPARγ in this process. Thus the study provides evidence that cyanidin-3-glycoside and its main intestinal metabolite protocatechuic acid might exert insulin-like activities by PPARγ activation, evidencing a causal relationship between this transcription factor and adiponectin and GLUT4 upregulation (Scazzocchio *et al.*, 2011).

6.1.3. Curcumin

Another polyphenolic flavonoid, is curcumin, found in turmeric is a yellow curry spice with a long history of use in traditional Indian diet and herbal medicine (Ammon and Wahl, 1991). Curcumin from *Curcuma longa* has many pharmacological activities including anti-inflammatory properties (Srimal and Dhawan, 1973), powerful antioxidant activity (Masuda *et*

al., 1999), anti-protease activity (Sui *et al.*, 1993), and cancer preventive properties (Kim *et al.*, 1998). Curcumin exerts anti-inflammatory and growth inhibitory effects in tumor necrosis factor (TNF)-α-treated HaCa T cells through inhibition of NF-κB and mitogen activated protein kinase pathways (Cho *et al.*, 2007). It has also been reported that curcumin exibhits an higher free radical scavenger activity when compared to vitamin E (Zhao *et al.*, 1989). Studies have shown that curcumin is a powerful scavenger of the superoxide anion, the hydroxyl radical, and nitrogen dioxide (Unnikrishnan and Rao, 1995) and that it also protects DNA against singlet-oxygen-induced strand breaks and lipids from peroxidation (Subramanian *et al.*, 1994). Oral administration of curcumin has been shown to be centrally neuroprotective (Rajakrishnan *et al.*, 1999) and displays protective effects in diabetic neuropathy (Sharma *et al.*, 2007). It also demonstrated neuroprotection against heavy metalinduced neurotoxicity (Dairam *et al.*, 2007). In diabetes, curcumin can suppress blood glucose levels (Arun and Nalini, 2002), increase the antioxidant status of pancreatic β-cells (Srivivasan *et al.*, 2003).

6.1.4.Theaflavins

They are natural polyphenols found in black tea, including theaflavin (TF1), theaflavin 3-gallate (TF2A), theaflavin 3'-gallate (TF2B), and theaflavin 3,3'-gallate (TF3) (Leung *et al.*, 2001). These tea polyphenols possess a broad spectrum of biological functions such as anti-oxidative, anti-bacterial, anti-tumour, anti-viral, anti-inflammatory and cardiovascular protection activities (Higdon and Frei, 2003, Mukhtar and Ahmad, 2000). In particular theaflavin 3,3'-gallate exerts protective effects against ischemia/reperfusion-induced brain injury by reducing oxidative stress and modulating the NF-κB activation. Acute stroke is a multi-component disorder, and its treatment will involve agents antagonizing multiple mechanisms. Therefore,

TF3, which acts as an antioxidant and redox modulator in a variety of systems, produces effective neuroprotection and represents a new class of naturally occurring agents with potential use in the therapy of human ischemic stroke (Cai *et al.*, 2007).

6.1.5.Quercetin

It is a bioflavonoid abundant in onions, apples, tea and red wine and one of the most studied flavonoids. It has been shown that quercetin scavenges reactive oxygen species and reduces oxidative DNA damage (Cai *et al.*, 1997). Quercetin treatment at three different dosages has decreased the stress-induced elevation of corticosterone. Various reports suggest that quercetin passes through the blood-brain-barrier and influences the neuronal cells directly. A higher concentration of quercetin metabolites appears in the brain after several hours of administration of quercetin (Day *et al.*, 2001, Paulke *et al.*, 2006). These quercetin metabolites might have influenced the swimming stress-induced activation of hypothalamicpituitary- adrenal axis. Observed decrease in corticosterone levels after quercetin treatment in stressed rats could be the result of quercetin action in the hypothalamus to decrease corticotrophin releasing factor (CRF). Quercetin inhibits lipid peroxidation and preserves the serum and hypothalamic levels of super oxide dismutase, catalase and glutathione peroxidase hence there is significant elevation in the antioxidant enzymes in the quercetin alone groups. Quercetin treatment caused the decrease in lipid hydroperoxides levels in the study as it can neutralize the reactive oxygen species by directly reacting with O_2^-, NO and peroxynitrite (Bongiovanni *et al.*, 2007, Muthukumaran *et al.*, 2008).

It has also been reported that quercetin metabolites can also inhibit peroxynitrite-mediated oxidation, similar to free quercetin and confirming that flavonoids can protect against

reactive oxygen species (Klotz and Sies, 2003). Therefore quercetin together with theaflavin, may act to prevent atherosclerosis through a combination of effects, including the inhibition of inflammation, the stimulation of NO, and the induction of HO-1.

Moreover quercetin has a preventive and protective effect in diabetes by decreasing oxidative stress and preservation of pancreatic ß-cell integrity Such damaged ß-cells often display extensive degranulation when examined histologically, and are clinically associated with the development of diabetes in some model animals for type 2 diabetes (Ihara *et al.*, 1999, Kaneto *et al.*, 1999)

6.2. Vitamin C

It stimulates cell metabolism, acting as a catalyst in cellular respiration and it is essential for the formation of collagen (Johnson *et al.*, 2003), blood, bones and teeth (Ting *et al.*, 1996). It regulates the turnover and enhances absorption of iron. It may serve in replacement of calcium, magnesium and zinc. Moreover vitamin C increases resistance to infectious diseases and contributes to recovery from physical fatigue. It may act as scavenger of nitrosamines (formed by nitrites and nitrates) (Barbosa *et al.*, 2008). Vitamin C or ascorbic acid is needed for proper leukocyte function and wound healing. Hyperglycemia increases urinary losses of ascorbate explaining why diabetic patients have low levels of vitamin C (Kajanachumpol *et al.*, 1997, Will and Byers, 1996). Low levels of ascorbic acid may make the diabetic patient more susceptible to wound infection, delayed healing, endothelial dysfunction, and tenosynovial disease. The normal amount to provide antioxidant protection (recommended dietary allowance) is 90 mg/day and 75 mg/day for men and women, respectively, and is based on the minimal urinary excretion of ascorbate (Johnson *et al.*, 2003). Since diabetic patients are deficient in vitamin C and have high

levels of retinoid esters in their lipoprotein particles (Wako *et al.*, 1986), the oxidation of their LDL-C may be increased and lead to an increase in foam cells. Providing the diabetic patient with enough vitamin C to cover losses in the urine and avoiding excessive retinoids in the diet may increase cellular antioxidant capacity and prevent oxidation and glycosylation exposure at the cellular level (Riccioni *et al.*, 2007).

6.3. Vitamin E

Its biological functions consist in counteracting, in synergy with GSH, the fatty acids peroxidation. This reaction produces tocopherol radicals that are neutralized by vitamin C and subsequent regeneration of vitamin E. It is also involved in the muscles and connective tissue development (Brigelius-Flohe and Traber, 1999). In synergy with vitamin C, it protects the skin from UVA and UVB radiations (Barbosa *et al.*, 2008). The recommended dietary requirement of vitamin E is 15 mg or about 20 units (35 μmol)/day (Johnson *et al.*, 2003).

Previous studies showed that vitamin E increased the incidence of subarachnoid hemorrhage but reduced the risk of ischemic stoke in a group of hypertensive men with diabetes without increasing the risk of hemorrhage (Ewy, 1999, Johnson *et al.*, 2003, Riccioni *et al.*, 2007), suggesting that vitamin E may reduce the risk of cardiovascular diseases and of microvascular complications in people with diabetes.

6.4. Beta carotene and Vitamin A

Carotenoids, in synergy with vitamin E and selenium, prevents lipid peroxidation of cell membranes inhibiting the radical peroxyles (Riccioni *et al.*, 2009). It contributes to the synthesis

of proteins, cell renewal, visual pigments formation and increases resistance to infection. It also prevents oxidation of vitamin C and acts in synergy with the complex of vitamins B, E, calcium and phosphorus. The optimal use of vitamin A requires the presence of alfa-tocopherol and zinc (Barbosa *et al.*, 2008).

6.5. Selenium

Selenium represents one of the major antioxidant trace elements. The most important physiological function of selenium in mammals is to form the catalytic site of the enzyme Se-GSHpx. Low Se-GSHpx activity is usually related to an increased risk of thrombosis, and a relationship between low serum selenium concentrations and an increased risk of cardiovascular death and myocardial infarction has been suggested (Neve, 1996). A selenium deficiency has not been clearly proved in diabetic patients. However most of the studies demonstrated a low Se-GSHpx in red blood cells and in platelets (Faure *et al.*, 1995). One of the advanced mechanisms is the susceptibility of Se-GSHpx to glycation. Under these conditions selenium need in diabetics should be should be highter than in the rest of the population. A possible mechanism involving selenium and the subsequent Se-GSHpx activity in vascular complications is the activation of the NF-κB (von *et al.*, 1999). It activation has been linked with the development of late diabetic macrovascular complications. Activation of NF-κB is induced by pro-oxidants (Li and Karin, 1999). On the other hand, various antioxidants inhibit NF-κB activation in response to different stressors (Bierhaus *et al.*, 1997). Hyperglycaemia directly activates NF-κB and promotes leukocyte adhesion to the endothelium and induces activation of NF-κB in vascular smooth cells (Yerneni *et al.*, 1999). Thus in the diabetic state, NF-κB can be activated through increased

oxidative stress but also by degradation of lipids or protein by free radical reaction. Patients with diabetic nephropathy exhibit higher NF-κB-binding activity in electrophoretic mobility shift assays compared to diabetic patients without renal complications (Hofmann *et al.*, 1998). In these conditions, through its role in lipid peroxidation, selenium supplementation could be efficient to reduce NF-kB activity and decrease vascular remodelling associated with diabetes.

6.6. Copper

It plays multiple biological functions including the intervention in the action of enzyme SOD, which converts free radicals into hydrogen peroxide. It is involved in the synthesis of phospholipids, in the production of ribonucleic acid (RNA), in the metabolism of vitamin C and tyrosine. It promotes bone growth and nervous system development. In red together collagen and elastin, for melanin production and energy metabolism. Sorption of copper is reduced by the presence of zinc (Hofmann *et al.*, 1998). Abnormal metabolism of Zn, Cu and Fe can lead to several chronic pathogenesis, such as diabetes overload. In the Fe and Cu overloading conditions, Fe and Cu chelating drugs could be used to control diabetes and diabetic complications (Zheng *et al.*, 2008).

6.7. Zinc

It performs several biological functions allowingthe enzymatic action of many enzymes. Along with copper, it potentiates the action of SOD which converts free radicals into hydrogen peroxide. It is involved in protein, and hormones synthesys, in growth processes, repair of tissue damage and immune defense. The presence of zinc is indispensable for the optimal metabolism of phosphorus, for the digestion of carbohydrates, for the synthesis of nucleic acid and the

absorption of vitamins (Haglund *et al.*, 1996, Zhao *et al.*, 2001). Epidemiological studies demonstrated that exposure to low concentrations of Zn in drinking water is associated with an increase of diabetes considering it as risk factor for the development of metabolic syndrome (Haglund *et al.*, 1996, Zhao *et al.*, 2001). Studies using animal models showed that various Zn chelators induce diabetes in some mammalian species, e.g. rabbits, mice, and hamsters, by ß-cell destruction, and that Zn deficiency significantly enhances the blood glucose level in diabetes-prone experimental animals (Kechrid *et al.*, 2001). Diabetes also significantly impairs Zn homeostasis. Terres-Martos et al. (Terres-Martos *et al.*, 1998) examined the status of serum Zn in 18 patients with diabetes and 326 compared it to healthy, age matched controls (Terres-Martos *et al.*, 1998). Serum Zn concentrations were significantly lower in diabetic patients than those in controls. In contrast, copper (Cu) levels were not significantly different from those in controls (Anderson *et al.*, 2001, Anetor *et al.*, 2002, Roussel *et al.*, 2003). Several studies, therefore, have examined whether Zn supplementation prevents systemic Zn deficiency and other general parameters of diabetic subjects. Two clinical studies found an improvement of systemic Zn and antioxidant status (Faure *et al.*, 1995, Kajanachumpol *et al.*, 1995). Zn supplementation significantly enhanced diabetes-decreased serum Zn level and antioxidant contents in blood cells. These studies were supported by subsequent clinical and animal studies (Uchiyama and Yamaguchi, 2003, Yamaguchi and Uchiyama, 2003). Therefore, all these observations strongly support the notion that Zn deficiency occurs in capacity .

6.8. Coenzyme Q10 (CoQ10)

It is an endogenous enzyme cofactor produced by most living cells in human (Crane, 2001). It is an essential component of the mitochondrial respiratory chain and exists in two

forms: ubiquinol (reduced) and ubiquinone (oxidized). Ubiquinol is a potent lipophilic antioxidant capable of recycling and regenerating other antioxidants such as tocopherol and ascorbate (Sohal, 2004). In human studies, plasma or serum CoQ10 concentrations have been regarded as a useful measurement of overall CoQ10 status (Bhagavan and Chopra, 2006). Previous study demonstrated that type 2 diabetes was associated with substantial (~40%) diminution in endothelial dependent and independent vasodilation and ~30% elevation in plasma of secretion of soluble intercellular adhesion molecule (sICAM) concentrations when compared to healthy control (Alam *et al.*, 2005, Caballero *et al.*, 1999, Luster, 1998, Widlansky *et al.*, 2003). Consistent with other report (Goldfine *et al.*, 2006), it was observed that endotelial dysfunction (ED) was already detectable among pre-diabetic subjects suggesting that the process of vascular injury set in early in the natural history of diabetic angiopathy (Goldfine *et al.*, 2006). In addiction it was also demonstrated that type 2 diabetes is associated with markedly decrease (approximately threefold) in plasma total CoQ10 concentrations. Intriguingly, it has been noted ubiquinone as the major species in type 2 diabetes rather than ubiquinol as in healthy subjects. It was hypothesized that these changes are related to on-going oxidative stress (Lim *et al.*, 2006). In recent years, clinical investigators began to explore the efficacy of oral CoQ10 in ameliorating type 2 diabetes associated ED (Playford *et al.*, 2003). Therefore, it has been hypothesized that oral CoQ10 replacement could restore the plasma concentrations in type 2 diabetes subjects thereby providing the substrate for normalization of ubiquinol/ubiquinone ratio. Furthermore, this favourable change may improve type 2 diabetes associated microcirculatory ED *in vivo*.

6.9. Melatonin

Its main biological functions, besides regulating sleep-wake cycles, are protection of cellular DNA from free radicals damage and stimulation of the immune system. It also contributes to the regeneration of connective tissues and protecting the cardiovascular system, it regulates the tone of the mood, improves the ability to deal stress and pathological states because it strengthens the effect of endorphins and lowers the level of aldosterone and cortisol (called "stress hormone"). Its antioxidant action is particularly pronunciated because of its effect on different types of free radicals, with an estimated strength of two folds compared to vitamin E (Li *et al.*, 1995). An increasing number of studies report that melatonin ameliorated diabetes- and chemically induced oxidative stress. The early studies of Montilla et al. showed that melatonin reduced hyperglycemia and hyperlipidemia in STZ-induced diabetic rats (Li *et al.*, 1995). When melatonin was injected 3 days before diabetes induction and daily thereafter for 8 week, it significantly increased oxidative parameters in the plasma and erythrocytes such as malonedialdehyde (MDA), reduced glutathione, glycemia, lipids, hemoglobin A1c (Hb A1c), and plasma fructosamine. With regard to the reductions in plasma HDL-c and GSH content of erythrocytes seen in these animals, melatonin returned their to normal levels. These results suggested that melatonin protected against oxidative stress and reduced the severity of STZ-induced diabetes. However, when administered after the induction of diabetes, melatonin had no effect on diabetic blood glucose levels, even though it reduced glucose levels when administered a few days before STZ injection. Melatonin was also shown to protect rat β-cells from the damaging effects of STZ. Melatonin treatment (200 μg/ kg/d, ip) for 3 days prior to the induction of diabetes and daily thereafter for 4 week restored islet morphology and β-cell insulin levels, and elevated the significantly reduced glutathione peroxidase activity in pancreatic tissue (Yavuz

et al., 2003). These data suggest that melatonin treatment may be potentially therapeutic in diabetics patients.

7. Antioxidants with enzymatic action

7.1. Superoxide Dismutase (SOD)

The enzyme SOD, represents an important antioxidant and catalyzes the following reaction:

$2\ O_2^- + 2\ H^+ \rightleftharpoons O_2 + H_2O_2$

They are proteins using copper and zinc as cofactors, or manganese, iron or nickel. All eukaryotic cells contain this enzyme SOD with copper and zinc (Cu-Zn-SOD). In humans, there are three isoforms of SOD. SOD1 is located in the cytoplasm, SOD2 in the mitochondria while SOD3 is extracellular (Li *et al.*, 1995). The first is a dimer, while the others are tetramers. The SOD1 and SOD3 contain copper and zinc, while SOD2 has manganese in its reaction center. The superoxide anion radical (O_2^-) turns spontaneously to O_2 e H_2O_2 fairly quickly. Nevertheless, the $O_2^{-\cdot}$ reacts even faster in the presence of groups such as $NO^{\cdot}$ and may form peroxynitrite. However, the SOD has an high turnover (speed of reaction with its substrate) compared to other enzymes, the reaction being limited only of the frequency of collision between it and O_2^-. Thus SOD catalyzes dangerous reaction of O_2^-, protecting the cell from the toxicity of this anion. The O_2^- is a major oxidizing agents in the cell and consequently, SOD plays a key antioxidant role. The physiological significance of SOD is evident by the serious illness in mice lacking these enzymes. Mice lacking SOD2 die in few days after birth, due to the strong oxidative stress (Li *et*

al., 1995). Those lacking SOD1 develop a wide variety of diseases, including hepatocellular carcinoma (Elchuri *et al.*, 2005), accelerating the loss of muscle mass related (Muller *et al.*, 2006), an early cataract and a lower life expectancy. Those who lack SOD3 do not show any evident defects, and have a normal life expectancy (Sentman *et al.*, 2006).

7.2. Catalase

Catalase is an oxidoreductase enzyme, involved in detoxification of the cell from ROS. Catalyzes the following reaction:

$2\ H_2O_2 \rightarrow\ O_2\ + H_2O$

in general, the enzyme catalyzes the conversion of hydrogen peroxide into H_2O and oxygen:

$4HO\cdot. \rightarrow O_2 + 2H_2O$

Hydrogen peroxide is a waste product of metabolism of many organisms. It is toxic because it can cause damage to membrane lipids, proteins and nucleic acids. Catalase is able to convert up to 5 million molecules of hydrogen peroxide per minute. The enzyme can also act, in some species, such as peroxidase, catalysing reactions such as the following:

ROOR' + electron donor molecule ($2e^-$) + 2H+ → ROH + R'OH

The lack or complete absence of catalase (linked to genetic defects in heterozygosity or homozygosity) may lead tovarious diseases, as occurs also with the deficiencies of other enzymes involved in detoxification of ROS. Defects dependent catalase could also play a role in amyotrophic lateral sclerosis (ALS), whose main genetic defect is dependent of the superoxide dismutase gene.

7.3. Heme Oxygenase

Heme oxygenase is a widely distributed enzyme in mammalian tissues, and its main function is associated with the degradation of heme to iron, carbon monoxide (CO), and biliverdin, the latter being converted to bilirubin by the cytosolic enzyme biliverdin reductase (Maines, 1997). Both biliverdin and bilirubin possess antioxidant properties, and HO-1-derived CO has been implicated in vasoregulation and signal transduction mechanisms (Clarke *et al.*, 2000, Kim *et al.*, 2006, Motterlini *et al.*, 1998). The HO-1 pathway appears to play a key role in the preservation of tissue integrity against oxidative stress, to contribute to the modulation of inflammatory responses in vivo, and to act in synchrony with other crucial enzymatic systems in the maintenance of cellular homeostasis (Foresti and Motterlini, 1999). In Alzheimer disease and mild cognitive impairment, immunoreactive HO-1 protein is over-expressed in neurons and astrocytes of the cerebral cortex and hippocampus relative to age-matched, cognitively intact controls and co-localizes to senile plaques, neurofibrillary tangles, and corpora amylacea. In Parkinson disease, HO-1 is markedly over-expressed in astrocytes of the substantia nigra and decorates Lewy bodies in affected dopaminergic neurons. HO-1 is also up-regulated in glial cells surrounding human cerebral infarcts, hemorrhages and contusions, within multiple sclerosis plaques, and in other degenerative and inflammatory human CNS disorders.

HO is also a target gene for the prevention of diabetes and obesity (Wallace, 1992), and, as seen in obese mice, an HO-1 apolipoprotein mimetic L-4F or cobalt compounds target HO-1 expression reduced visceral and subcutaneous adiposity, increased adiponectin levels, and improved insulin sensitivity (Charonis *et al.*, 1990).

In a recent study (Abraham *et al.*, 2003), it was demonstrated that increased expression of HO-1 attenuates glucose-mediated cell growth arrest and reduces apoptosis in human microvessel

endothelial cells. The protective effect of the HO-1 signaling mechanism, which attenuated glucose-mediated endothelial cell apoptosis, was maintained through suppression of p21 and p27 cyclin kinase inhibitors or, probably (Abraham *et al.*, 2003), through its powerful antioxidant properties.

8. Perspective

The many studies on oxidative stress, antioxidant treatment, and diabetic complications have shown that oxidative stress is increased and may accelerate the development of complications linked to the metabolism of excessive glucose and insulin-resistant states. The new therapeutic approaches for the prevention and treatment of diabetic complications, are all based on the new paradigm of a unifying mechanism for the pathogenesis of diabetic complications or increased oxidative stress as mentioned. Many different types of antioxidants such as Polyphenols, vitamin E, β-carotene, HO-1,SOD, catalase and others have been studied in animal models of diabetes and in diabetic patients (Doi *et al.*, 2001, Haak *et al.*, 2000, Kowluru and Kennedy, 2001, Mooradian, 2003, Packer *et al.*, 2001, Ruhe and McDonald, 2001). It has been reported that antioxidants prevented hyperglycemia-induced biological changes including cytokine expression, matrix synthesis, and cellular growth and turnover (Greene *et al.*, 2001, Montero *et al.*, 2000, Studer *et al.*, 1997, Trachtman *et al.*, 1993). There is mounting evidence to support the notion that antioxidants such as those listed above can prevent, and even reverse, many early changes in the neurological tissues from diabetic animals (Abiko *et al.*, 2003, Koya *et al.*, 1997).

Antioxidants may eliminate free radicals toxic effects, as confirmed by evidence that people consuming fruits and vegetables rich in polyphenols and anthocyanins have a lower risk of serious complications following hyperglycaemia (53, 54). In conclusion, diabetic treatment would most likely be more effective if it is coupled with antioxidant treatments. Thus, antioxidant molecules could be useful as protective agents against oxidative stress during pharmacological therapy of diabetes.

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Table 1. Complications related to diabetes

Complications	Descriptions
Diabetic nephropathy	it is a progressive reduction of the filter function of the kidney that, if untreated, can lead to renal failure up to the need of dialysis and/or kidney transplant.
Cardiovascular disease	the risk of cardiovascular disease is 2 to 4 times higher in people with diabetes than in the rest of the population, causing, in industrialized countries, more than 50% of deaths in diabetic patients. This leads us to consider the cardiovascular risk in diabetic patients equal to that given to a patient who had a cardiovascular event.
Diabetic neuropathy	is one of the most frequent complications and according to the World Health Organization is manifested at different levels in 50% of diabetics. It can cause loss of sensitivity, pain of varying intensity, damage to limbs, requiring amputation in more severe cases, micro and macroangiopaties with an increase in vascular permeability. It may involve heart dysfunction of the, eyes, stomach and is a major cause of male impotence.
Diabetic foot	the alteration of the structure of blood vessels and nerves can cause ulcers and problems at the level of the lower limbs, especially of the foot. This may necessitate the amputation of limbs and statistically is the most common cause of amputation of the lower limbs of non-traumatic origin(3)
Complications during pregnancy	in pregnant women, diabetes can result in adverse effects on the fetus from birth defects to a high birth weight, up to a high risk of perinatal mortality(2)
Diabetic retinopathy	is a damage to the small blood vessels that serve the retina, with loss of visual ability. In addition, people with diabetes have more probability to develop eye diseases such as glaucoma and cataracts.

Table 2. Potential mechanisms underlying the diabetes-related changes in the blood–brain barrier

1. Pathological changes in cerebral microcirculation	a) decreased capillary density b) increased basement membrane thickness
2. Hemodynamic changes in cerebral microcirculation	a) arteriovenous shunting b) altered vascular reactivity
3. Biophysical changes in cerebral capillaries	a) altered membrane fluidity b) altered surface charge of endothelium
4. Biochemical changes in cerebral capillaries	a) altered protein composition b) altered lipid composition c) accumulation of lipid peroxidation byproducts
5. Alterations in neurotransmitter activity in cerebral capillaries	a) altered beta-adrenergic receptor number b) altered adenylate cyclase activity

Figure Legends

Figure 1. The Polyol Pathway Flux.

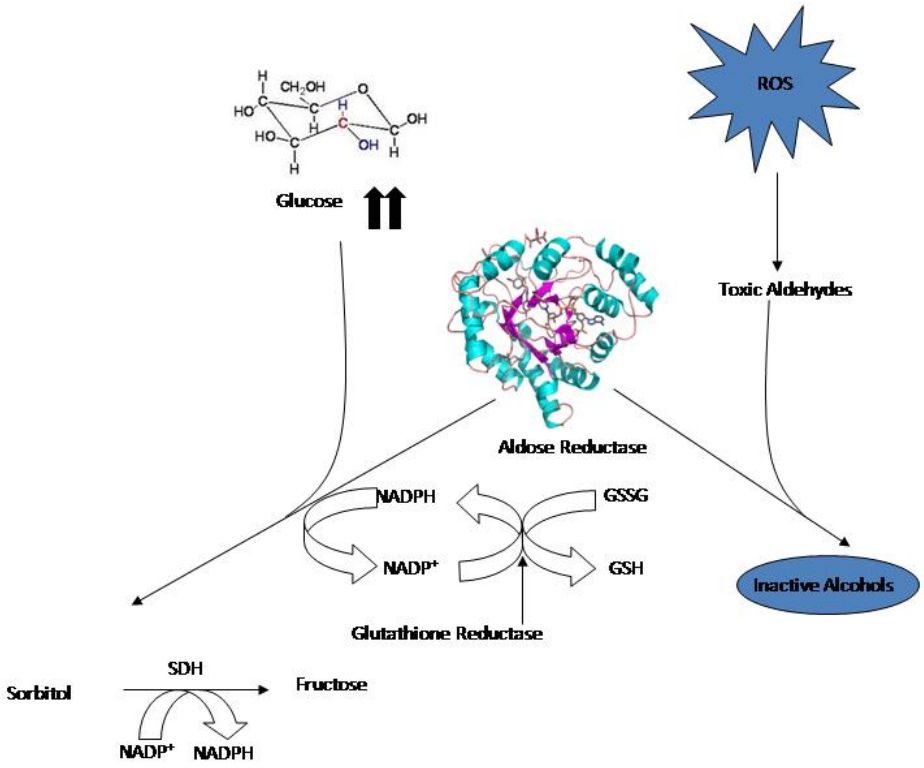

Figure 1

Figure 2. Mechanisms by which intracellular production of advanced glycation end-product (AGE) precursors damages vascular cells.

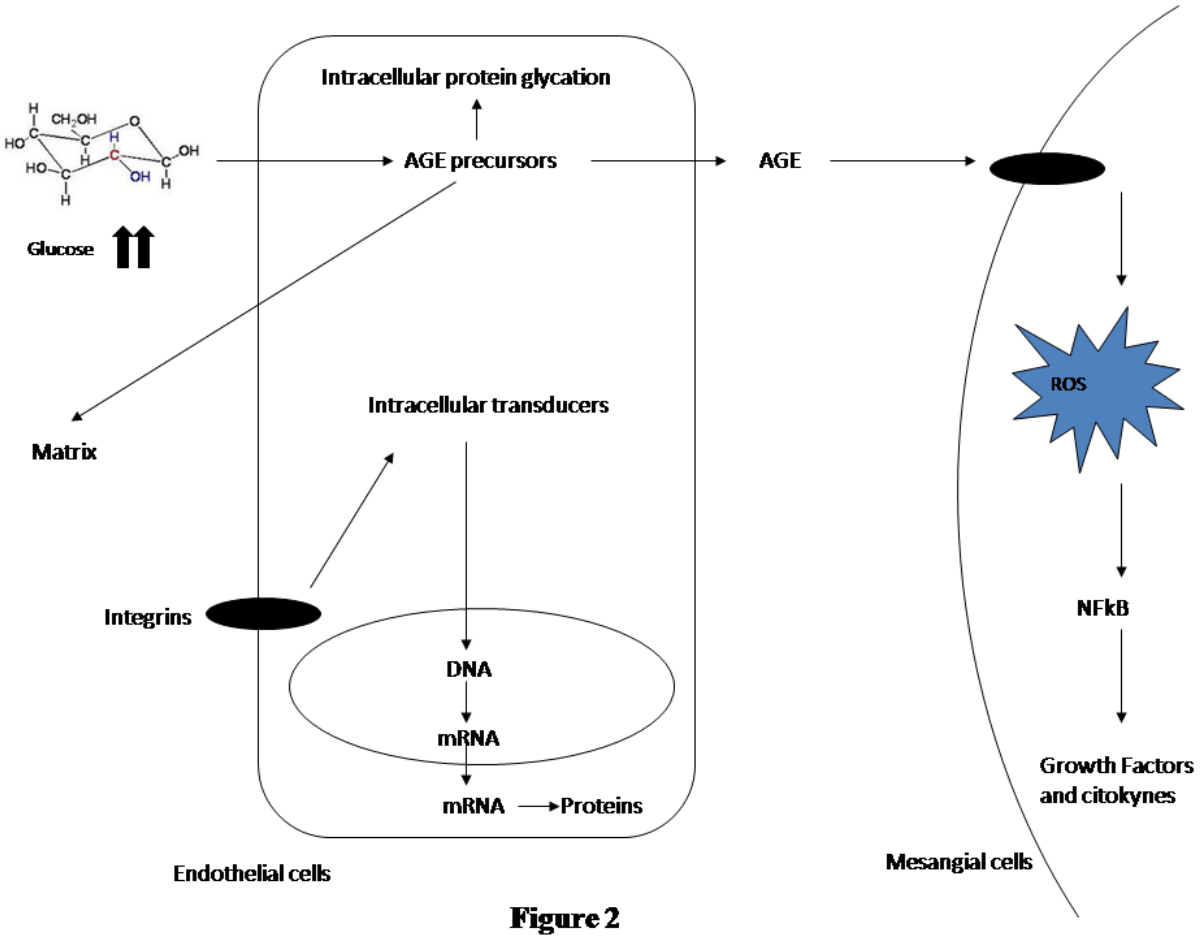

Figure 2

Figure 3. Consequences of hyperglycaemia-induced activation of protein kinase C (PKC).

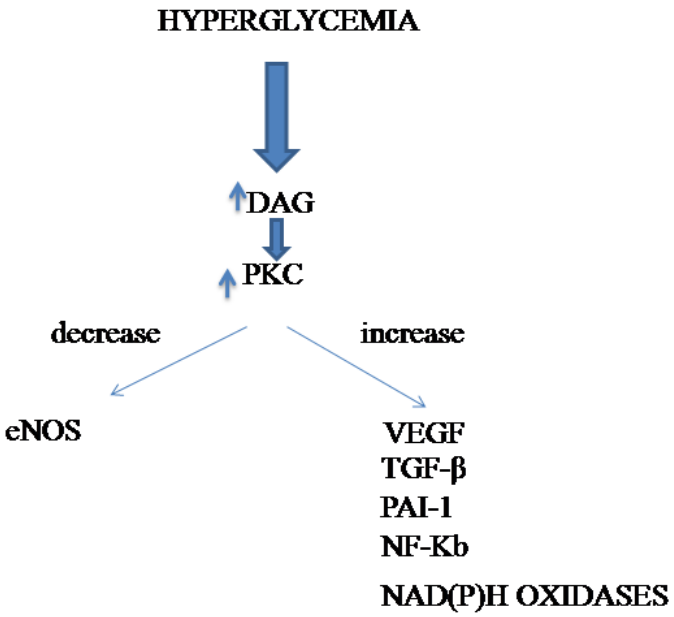

Figure 3

Figure 4. The hexosamine pathway.

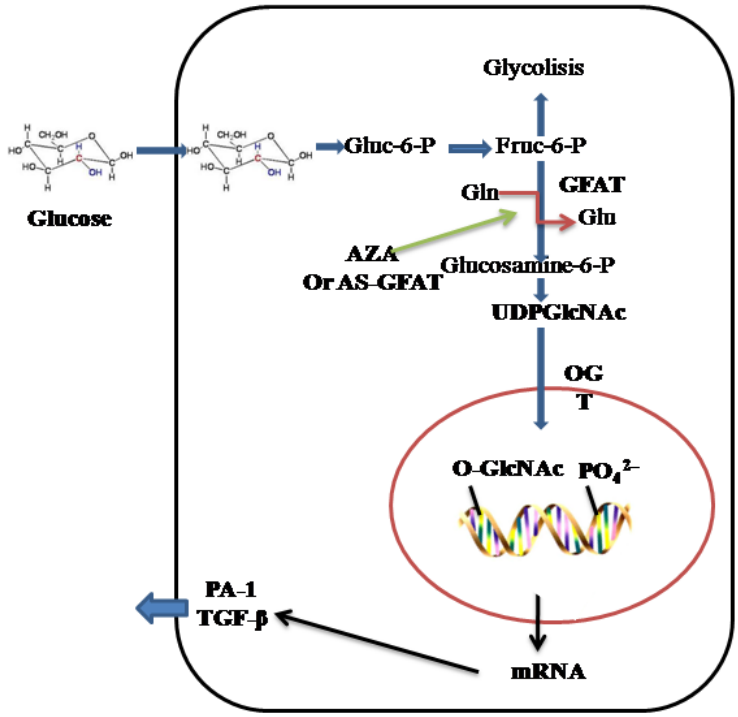

Figure 4

Figure 5. Production of superoxide by the mitochondrial electron-transport chain.

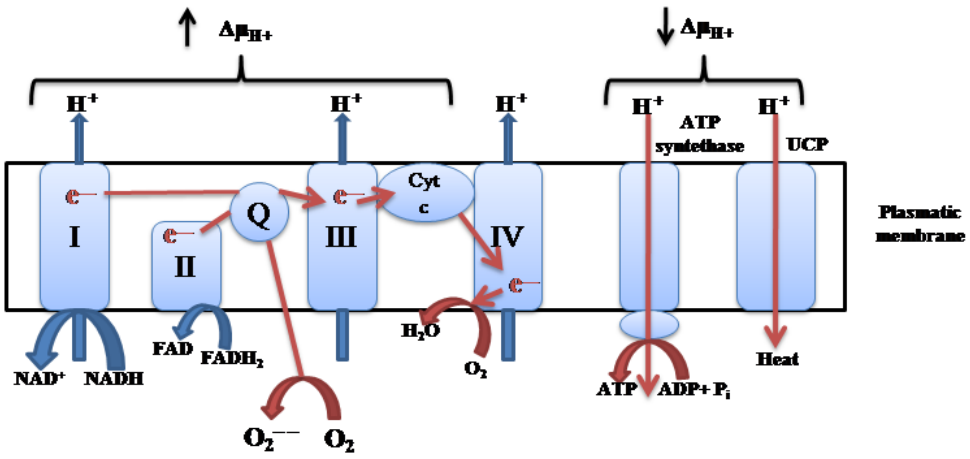

Figure 5

Figure 6. The superoxide (O_2^-) by reacting with nitric oxide (NO) produces peroxynitrite ($ONOO^-$).

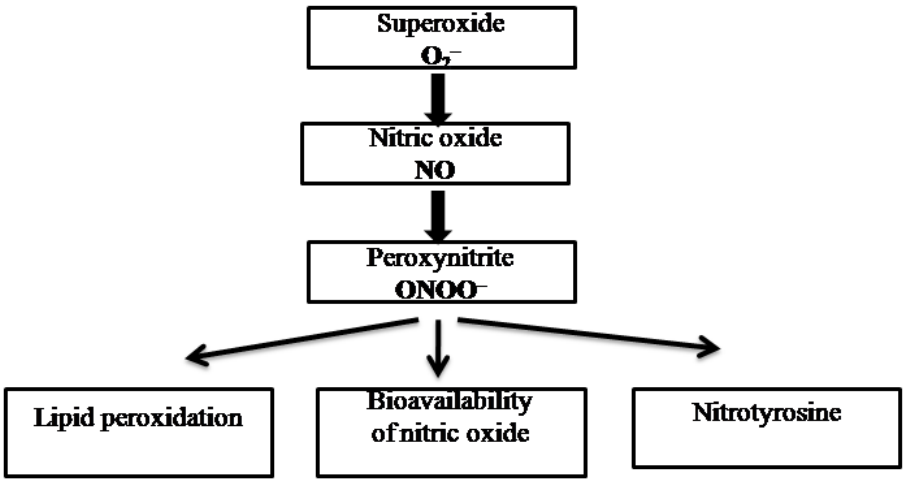

Figure 6

Figure 7. The hyperglycaemia leads to an overproduction of superoxide through the chain of electron transport mitochondrial and promotes the increased expression of NADPH and iNOS through the activation of NF-kB.

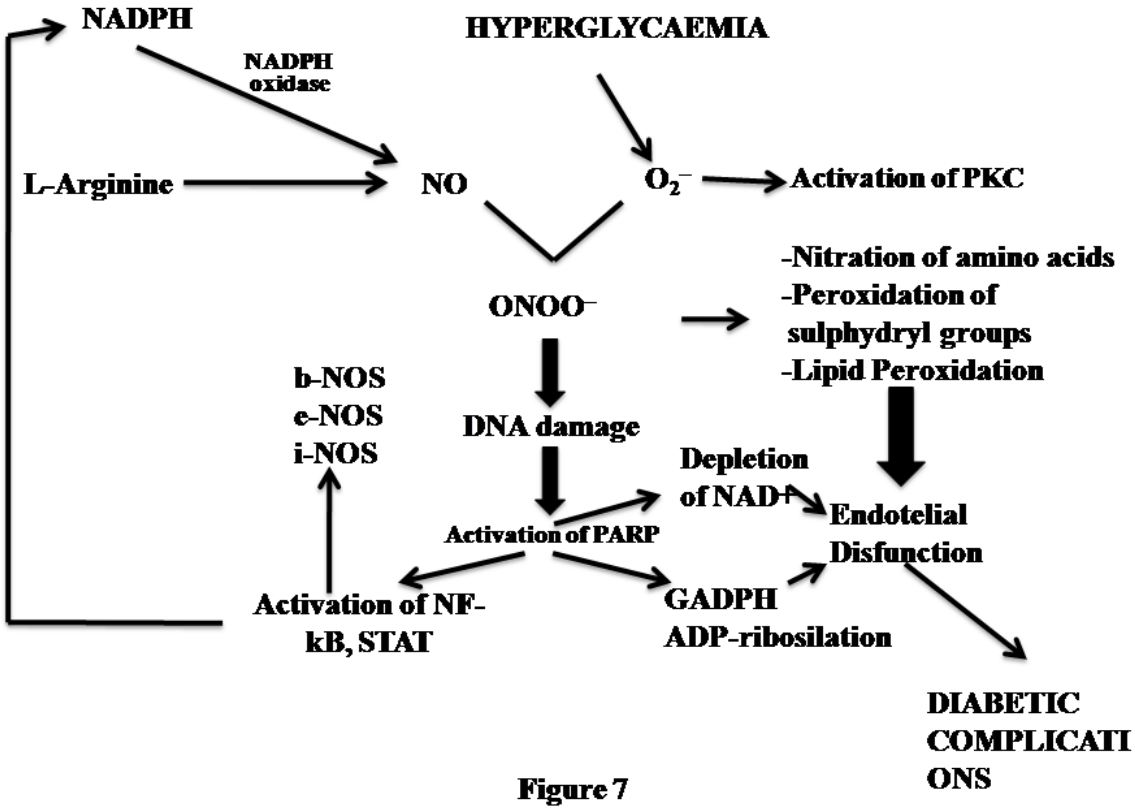

Figure 7