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Efeito do Consumo Diário de Chá Branco no Córtex Cerebral de Ratos Pré-diabéticos

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O conteúdo do presente trabalho é da exclusiva responsabilidade do autor:

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(Ana Raquel Mendes da Paula Nunes)

"Acredita no melhor... tem um objetivo para o melhor, nunca fiques satisfeito com menos que o teu melhor, dá o teu melhor, e a longo prazo as coisas correrão pelo melhor."

(Henry Ford)

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Resumo

A Diabetes Mellitus (DM) é um problema de saúde pública e a sua incidência está a aumentar drasticamente. O cérebro, especialmente o córtex cerebral, é muito suscetível a flutuações dos níveis de glucose e ao stress oxidativo induzido pela hiperglicémia. O chá (*Camellia sinensis* (L.)) é amplamente consumido, porém as propriedades antidiabéticas e neuroprotetoras do chá branco permanecem por explorar. Neste trabalho investigámos os efeitos do consumo diário de chá branco no córtex cerebral de ratos pré-diabéticos. Os animais foram divididos aleatoriamente em três grupos: grupo controlo, grupo de ratos pré-diabéticos que ingeriram água e grupo de ratos pré-diabéticos que ingeriram chá branco. O perfil metabólico do córtex foi avaliado e a expressão dos níveis dos transportadores de glucose (GLUTs), da fosfofrutoquinase-1, da lactato desidrogenase (LDH) e do transportador de monocarboxilato 4 foram também determinados. O perfil oxidativo do córtex foi obtido através da avaliação do seu poder antioxidante (ensaio FRAP) e dos níveis de peroxidação lipídica (ensaio TBARS) e de oxidação proteica (ensaio dos grupos carbonilo). A catalase e a glutationa, bem como o conteúdo em glutamato, N-acetilaspartato, aspartato, colina, ácido gama-aminobutírico, taurina e valina foram também determinados. Embora o consumo diário de chá branco não tenha diminuído os níveis de glicémia no sangue, melhorou a tolerância à glucose e a sensibilidade à insulina dos ratos pré-diabéticos. Além disso, o consumo diário do chá branco alterou o perfil glicolítico do córtex cerebral dos ratos pré-diabéticos, modulando a expressão dos GLUTs e o conteúdo em lactato e alanina. O consumo de chá branco também foi capaz de restabelecer os níveis de oxidação proteica e de peroxidação lipídica para valores controlo, no córtex de ratos pré-diabéticos. No geral, o córtex cerebral de ratos pré-diabéticos que consumiram chá branco apresentou uma maior capacidade antioxidante e uma expressão normalizada da catalase. Concluindo, o consumo diário de chá branco por ratos pré-diabéticos melhora o perfil metabólico e oxidativo do seu córtex cerebral, sugerindo que a ingestão de chá branco pode ser uma estratégia boa, segura e económica para evitar os efeitos relacionados com a DM no córtex cerebral.

Palavras-chave

Camellia sinensis, Chá Branco, Córtex Cerebral, Pré-diabetes, Metabolismo, Antioxidantes.

Resumo Alargado

A Diabetes Mellitus (DM) representa uma das maiores ameaças à saúde pública nas sociedades modernas. A sua incidência tem vindo a aumentar drasticamente e, de acordo com a Organização Mundial de Saúde, estima-se que esta doença vá afetar cerca de 300 milhões de pessoas em 2025. Estes números tendem a agravar-se devido a alguns fatores de risco relacionados com o estilo de vida, tais como o excesso de peso, a dieta desequilibrada ou o hábito de fumar. A DM é uma doença metabólica caracterizada por hiperglicémia resultante de defeitos na secreção e/ou ação da insulina. A doença pode ser dividida em dois tipos: a DM tipo 1 (T1DM) e a DM tipo 2 (T2DM). A T1DM tem geralmente o seu desenvolvimento numa idade jovem, e é causada pela destruição autoimune das células beta pancreáticas, pelo que requer uma terapia diária de reposição de insulina. Por sua vez, a T2DM é a forma mais comum da doença, sendo responsável por 95% de todos os casos de DM. Atualmente, afeta crianças, adolescentes e adultos jovens que irão enfrentar o fardo da doença por períodos prolongados. A T2DM ocorre quando as células beta pancreáticas não conseguem produzir insulina suficiente para manter os níveis de glucose no sangue dentro dos parâmetros normais.

O diagnóstico da DM é complexo, o que levou a que fosse estabelecido um estado intermédio denominado “pré-diabetes”. O estado pré-diabético antecede o aparecimento da doença propriamente dita, e a sua prevalência está a aumentar entre os jovens. Contudo, pode ser reversível. A intolerância à glucose, bem como a resistência à insulina e os níveis de glucose no sangue acima do normal estão associados a este estado. Indivíduos pré-diabéticos têm importantes alterações metabólicas que propiciam a progressão para T2DM. A transição do estado pré-diabético para T2DM ocorre quando a capacidade secretora das células beta pancreáticas não é capaz de compensar a resistência à insulina.

O comprometimento do metabolismo da glucose, tanto numa fase inicial como numa fase mais tardia da diabetes, afeta vários órgãos, incluindo o cérebro. Sabe-se que o cérebro utiliza a glucose como a sua principal fonte de energia e, assim, é expectável que a disfunção no metabolismo da glucose leve a danos cerebrais graves. Na verdade, a hiperglicémia, mesmo que transitória, pode provocar efeitos deletérios sobre a função cerebral. Elevados níveis de glucose sanguínea afetam, de modo diferente, várias regiões do cérebro, sendo o córtex cerebral particularmente sensível. Tem sido descrito que os neurónios corticais e os astrócitos são mais vulneráveis à desregulação do metabolismo glicolítico do que as células do corpo estriado ou do hipocampo. Por outro lado, o córtex apresenta reduzidas defesas antioxidantes, tornando-o muito suscetível ao stress oxidativo

(OS). O OS ocorre quando existe um desequilíbrio entre a formação de espécies reativas de oxigénio (ROS) e a proteção contra as mesmas. Embora as ROS sejam importantes em diversos processos biológicos, quando presentes em excesso, causam danos severos nos ácidos nucleicos, nos lípidos, nas membranas celulares e nas proteínas. Assim, é importante manter os níveis de ROS controlados. Além disso, o córtex cerebral é afetado em várias doenças neurodegenerativas, pelo que a hiperglicémia pode desempenhar um papel importante no desenvolvimento e progressão das mesmas.

A DM é uma doença incurável e a limitação das terapias existentes para o seu tratamento e das suas complicações, tem encorajado a procura de alternativas mais eficientes, associadas a alterações na dieta e estilos de vida. Nos últimos anos, é notório o aumento crescente do interesse pelos produtos nutracêuticos, como complemento ou até mesmo substituição de terapias atuais para a prevenção e tratamento das mais diversas patologias. As plantas medicinais (e respetivos extratos) são usadas, desde os tempos mais antigos, na redução dos níveis de glicémia. Contudo, os mecanismos através dos quais exercem esse efeito no organismo ainda não foram totalmente elucidados.

O chá (*Camellia sinensis* (L.)) é uma das bebidas mais consumidas a nível mundial, e é composto por vários componentes bioativos, que têm sido descritos como promotores de benefícios para a saúde. Propriedades antioxidantes, antidiabéticas e neuroprotetoras têm sido atribuídas aos compostos fenólicos encontrados no chá, especialmente às catequinas. Por outro lado, tem sido descrito que o consumo de chá interage com várias vias metabólicas, suprimindo a resistência à insulina e melhorando a sensibilidade à mesma. Dos vários tipos de chá existentes, o chá branco é dos menos estudados. Este chá é preparado a partir dos rebentos e folhas imaturas da *C. sinensis*, contendo níveis de antioxidantes mais elevados comparativamente aos outros tipos de chá.

O objetivo a que nos propusemos neste trabalho foi o de estudar os efeitos do consumo diário de chá branco no córtex cerebral de um modelo animal de pré-diabetes induzido por estreptozotocina (STZ). Pretendeu-se avaliar de que modo a ingestão deste tipo de chá influencia o perfil metabólico e oxidativo do córtex cerebral. Para o efeito foram usados três grupos experimentais de ratos macho Wistar (*Rattus norvegicus*): um grupo controlo, um grupo de pré-diabetes induzida por STZ com consumo diário de água, e um grupo de pré-diabetes induzida por STZ mas com consumo diário de chá branco em vez de água. Foi avaliado o perfil metabólico e os níveis de expressão dos transportadores de glucose (GLUTs), da fosfofrutoquinase-1, da lactato desidrogenase (LDH) e do transportador de monocarboxilatos do tipo 4. A atividade da LDH foi também avaliada. O perfil oxidativo do córtex cerebral foi determinado através da avaliação do seu poder antioxidante (ensaio FRAP) e dos níveis de peroxidação lipídica (TBARS) e oxidação proteica (ensaio dos grupos carbonilo). A catalase e a glutationa, bem como o conteúdo em glutamato, N-

acetilaspartato, aspartato, colina, ácido gama-aminobutírico, taurina e valina foram também determinados.

Os nossos resultados mostram que foi possível desenvolver um modelo de pré-diabetes adequado, com as características que caracterizam esta situação. Embora o consumo diário de chá branco não tenha diminuído os níveis de glicémia no sangue, foi capaz de melhorar a tolerância à glucose e a sensibilidade à insulina dos ratos pré-diabéticos. Por outro lado, a ingestão deste tipo de chá alterou o perfil glicolítico do córtex cerebral de ratos pré-diabéticos, através da modulação da expressão dos GLUTs e do conteúdo em lactato e alanina. Os níveis de oxidação proteica e de peroxidação lipídica foram restaurados para os valores controlo. O córtex de ratos pré-diabéticos que consumiram o chá branco apresentaram uma maior capacidade antioxidante e uma expressão normalizada da catalase.

Em conclusão, o consumo diário de chá branco por ratos pré-diabéticos melhora o perfil metabólico e oxidativo do seu córtex cerebral. Deste modo, a ingestão regular deste tipo de chá parece ser uma estratégia segura e económica de evitar os efeitos relacionados com a DM no cérebro, nomeadamente a nível do córtex cerebral.

Abstract

Diabetes Mellitus (DM) is a major public health problem and its incidence is dramatically rising. The brain, particularly the cerebral cortex, is very susceptible to glucose fluctuations and hyperglycemia-induced oxidative stress. Even though tea (*Camellia sinensis* (L.)) is widely consumed, white tea antidiabetic properties remain largely unexplored. Herein, we investigated the effects of white tea daily consumption on the cerebral cortex of prediabetic rats. Animals were randomly divided in 3 groups: control and prediabetic rats drinking water or white tea. Cortex metabolic profile was evaluated and expression of glucose transporters (GLUTs), phosphofructokinase-1, lactate dehydrogenase (LDH), monocarboxylate transporter 4 levels was assessed. LDH activity was also determined. Cortex oxidative profile was determined by evaluating its antioxidant power, lipid peroxidation and protein oxidation levels. Catalase, glutathione, glutamate, N-acetylaspartate, aspartate, choline, gamma-aminobutyric acid, taurine and valine contents were determined. Daily white tea consumption ameliorated glucose tolerance and insulin sensitivity and altered the cortex glycolytic profile of prediabetic rats by modulating GLUTs expression and lactate and alanine contents. It also restored protein peroxidation levels, catalase expression and improved antioxidant capacity of the cortex of prediabetic rats. In conclusion, daily white tea consumption by prediabetic rats improves cerebral cortex metabolic and oxidative profile suggesting that it can be a good, safe and inexpensive strategy to prevent DM-related effects in the cerebral cortex.

Keywords

Camellia sinensis, White tea, Cerebral Cortex, Prediabetes, Metabolism, Antioxidants.

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List of Abbreviations

^{1}H NMR - Proton Nuclear Magnetic Resonance

Acetyl-CoA – Acetyl coenzyme A

AD – Alzheimer's disease

ADA – American Diabetes Association

AGEs – Advanced glycation end-products

ALT – Alanine aminotransferase

ATP – Adenosine triphosphate

AUC – Area under the curve

AUC_{GTT} - Area under the curve for glucose tolerance test

AUC_{ITT} - Area under the curve for insulin tolerance test

BBB – Blood-brain barrier

BSA - Bovine serum albumin

CNS – Central nervous system

DM – Diabetes Mellitus

DNP - 2,4-Dinitrophenol

DPPH - Diphenylpicrylhydrazyl

DTNB - 5,5'-dithiobis-(2-nitrobenzoic acid)

EC – (-)epicatechin

ECF - Enhanced chemifluorescence

ECG – (-)-epicatechin-3-gallate

EGC – (-)-epigallocatechin

EGCG – (-)-epigallocatechin-3-gallate

ETC – Electron transport chain

FRAP - Ferric reducing antioxidant power

GABA - Gamma-aminobutyric acid

GLUT1 – Glucose transporter 1

GLUT3 – Glucose transporter 3

GLUTs - Glucose transporters

GSH - Reduced glutathione

GSSH - Oxidized glutathione

GTT - Glucose tolerance test

HK – Hexokinase

IFG – Impaired fasting glucose

IgG-AP - Alkaline phosphatase linked immunoglobulin G

IGT – Impaired glucose tolerance

INT - Tetrazolium salt

IP - Intraperitoneal

ITT - Insulin tolerance test

LDH – Lactate dehydrogenase

MCT4 – Monocarboxylate transporter 4

MCTs – Monocarboxylate transporters

MDA - Malondialdehyde

NAA - N-Acetylaspartate

NAD^+ - Oxidized nicotinamide adenine dinucleotide

NADH - Reduced nicotinamide adenine dinucleotide

NADPH – Nicotinamide adenine dinucleotide phosphate

OADs - Oral antidiabetic drugs

OS – Oxidative stress

PBS - Phosphate buffered solution

PD – Parkinson's disease

PFK-1 – Phosphofructokinase 1

PO – Polyphenol oxidase

PrDM - Prediabetes

PSMF - Phenylmethylsulfonyl fluoride

RNS – Reactive nitrogen species

ROS – Reactive oxygen species

SSA - 5-sulfosalicylic acid

STZ - Streptozotocin

T1DM – Type 1 diabetes mellitus

T2DM – Type 2 diabetes mellitus

TBA - Thiobarbituric acid

TBARS - Thiobarbituric acid reactive species

TCA – Tricarboxylic acid

TNB - 2-nitro-5-mercaptobenzoic acid

TP – Tea polyphenols

TPTZ - 2,4,6-Tripyridyl-s-Triazine

WHO – World Health Organization

WTEA - White tea

I. Introduction

1. Diabetes Mellitus at a Glance

Diabetes Mellitus (DM) represents one of the greatest threats to modern global health and its incidence is rapidly increasing. The World Health Organization (WHO) estimated that about 300 million of people will develop DM in 2025 (Agbaje, I. M. et al., 2007). These numbers tend to aggravate due to some risk factors related to lifestyle, such as being overweight, having an unhealthy diet or smoking. Nowadays, this disease is considered one of leading causes of morbidity and mortality in both developed and under development countries. It is potentially devastating, and although treatable, it is a lifelong disease (Al-Attar, A. M. and Zari, T. A. 2010). Thus, the healthcare costs associated with DM are enormous (Zhang, P. et al., 2010).

DM is described as a metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia that can result from defects in insulin secretion and/or insulin action (Association, A. D. 2010). Moreover, there is a severe alteration in carbohydrate, lipid, protein metabolism (Association, A. D. 2010) and defects in reactive species of oxygen (ROS) scavenging enzymes (Kesavulu, M. M. et al., 2000), which results in increased oxidative stress (OS) and impairment of the pancreatic beta cells (Kahn, S. E. 2001). The DM may be classified as Type 1 Diabetes Mellitus (T1DM) or Type 2 Diabetes Mellitus (T2DM). T1DM is responsible for only 5-10% of those with DM and generally develops at young age with the great majority of the patients being diagnosed before the age of 30 (Agbaje, I. M. et al., 2007). It results from the autoimmune destruction of the insulin-producing beta pancreatic cells, and therefore there is a complete lack of insulin that leads to the increase of glucose levels in blood and urine (Association, A. D. 2010). Thus, T1DM patients need exogenous insulin administration and are insulin dependent. Untreated T1DM is characterized by hyperglycemia, hypoinsulinemia, ketonuria, and hyperlipidemia, resulting from a general metabolic failure (for review see (Emilien, G. et al., 1999)). In turn, T2DM is the most common type of DM, accounting for up to 90-95% of all cases diagnosed (Association, A. D. 2010). Among other features, T2DM is characterized by insulin resistance and/or insufficient insulin secretion. As a result, body glucose metabolism becomes compromised. The risk of developing T2DM increases with age, obesity, cardiovascular diseases and lack of physical activity (for review see (Golay, A. and Ybarra, J. 2005)). Noteworthy, the clinical symptoms are frequently detected only in an advanced phase of the disease, allowing the progression of functional changes in cells and tissues that may not be reverted. Diabetic patients possess a higher risk of death, together with lower survival rates and lower life expectancy than non-diabetic persons (Gu, K. et al., 1998).

DM is an incurable disease but there are many strategies available for its treatment such as the stimulation of endogenous insulin secretion, enhancement of insulin action at

the target tissues, inhibition of dietary starch lipid degradation, and pharmacological treatment with oral antidiabetic drugs (OADs) like biguanides (e.g. metformin) and sulfonylureas (e.g. clorpropamid) (Birari, R. B. and Bhutani, K. K. 2007, García-Pérez, L. E. et al., 2013)). However, these OADs can cause side effects like major and minor hypoglycemia, gastrointestinal problems, peripheral edema, body weight gain, liver diseases and, over time, they lose their efficacy (Dilla, T. et al., 2008, Donnelly, L. A. et al., 2009, García-Pérez, L. E. et al., 2013).

The limitation of existing therapies for the treatment of diabetes has encouraged the search of more efficient and cost-effective alternatives, recurring to dietary and lifestyle changes. In recent years, there is an increased interest in functional and nutraceutical food for pharmacological purposes, in order to complement or replace current therapies. It has been reported that numerous extracts obtained from plants can be used in DM treatment to reduce glycemia (Gupta, R. K. et al., 2005, Abolfathi, A. A. et al., 2012). However, little is known about the molecular mechanisms involved when using such extracts.

1.1 Prediabetes: A Prodromal Stage of DM

The complexity of DM diagnosis, especially in obese patients, led to the establishment of an intermediate state known as "prediabetes". Prediabetes is defined as elevated blood glucose levels, although not sufficient to meet the criteria for established diabetes (Association, A. D. 2010). The American Diabetes Association (ADA) defined prediabetes as either impaired fasting glucose (IFG) (100-110 mg/dL) and/or impaired glucose tolerance (IGT) (140-199 mg/dL) (Association, A. D. 2010). The prediabetic state is characterized by resistance to insulin-mediated glucose disposal and compensatory hyperinsulinemia (Reed, M. J. et al., 2000, Alves, M. G. et al., 2013a) and its prevalence is increasing among young people (Association, A. D. 2010). This intermediate state is commonly associated with the metabolic syndrome which represents a group of abnormalities, including overweight (visceral abdominal fat distribution), dyslipidaemia, hypertension, and impaired glucose metabolism, with insulin resistance as the postulated underlying pathogenic mechanism (for review see (Kasturi, S. S. and Tannir, J. 2008)). Prediabetic patients have important metabolic alterations that increase the risk for the development of T2DM (for review see (Engelgau, M. M. et al., 2000)). The transition from prediabetes to T2DM occurs when the secretory capacity of the pancreatic beta cells is no longer able to compensate insulin resistance. This progression occurs over many years and compelling evidence support that intervention delay the progression from prediabetes to DM (Weyer, C. et al., 1999).

Glycemic levels are rapidly increasing in developed and developing countries, which increases the prevalence of prediabetes and it is projected that more than 470 million people will have prediabetes in 2030 (for review see ((Tabák, A. G. et al., 2012)). Every year, about 5–10% of the individuals with prediabetes become diabetic (Nathan, D. M. et al., 2007) and population habits may increase these rates.

The prediabetic state is not only related to an increased risk of DM development and its complications. Damage on kidney and nerves occurs in these individuals (Fox, C. S. et al., 2005). Besides, prediabetes can lead to complications such as nephropathies and chronic kidney disease, neuropathies, diabetic retinopathy, and macrovascular diseases (for review see (Tabák, A. G. et al., 2012)). Along with these complications, the risk of cognitive decline and neurodegeneration are increased in these patients (Luchsinger, J. A. et al., 2004). The exact pathophysiology of alterations that occur in the brain of prediabetic subjects is not completely understood, but it is likely that abnormal levels of blood glucose and insulin resistance play significant roles (for review see (Kodl, C. T. and Seaquist, E. R. 2008)).

2. General Effects of Hyperglycemia on the Brain

Blood glucose concentrations alter the function of several organs and tissues. As discussed above, DM is a complex metabolic disorder and hyperglycemia is a hallmark of this disease as a consequence of impaired insulin synthesis and/or insulin resistance. Consequently, glucose is not efficiently transported and metabolized in the target organs. Chronic hyperglycemia is associated with long-term injury and dysfunction of several organs, including a slow progressive brain damage (Diaz-Parejo, P. et al., 2003, Biessels, G. J. and Gispen, W. H. 2005).

The mammalian brain depends upon glucose as its main source of energy. Thus, a tight regulation of glucose metabolism is critical for brain physiology. In recent years, significantly more interest has been dedicated to the effect of hyperglycemia on the brain. DM is implicated in the development of cerebrovascular disease and other neurological co-morbidities, such as cognitive dysfunction and dementia (for review see (Roriz-Filho, J. S. et al., 2009)). A study conducted by Luchsinger and collaborators (2004) suggested that the risk of cognitive decline and neurodegeneration are increased in prediabetic patients. The exact pathophysiology of brain damage caused by DM is not completely understood, but it is likely that hyperglycemia and insulin resistance play a significant role (for review see (Roriz-Filho, J. S. et al., 2009)). Changes in peripheral insulin and glucose homeostasis may affect the action of insulin on the brain and its receptors functions (for review see (Gasparini, L. and Xu, H. 2003)). Moreover, insulin resistance leads to formation of

advanced glycation end-products (AGEs) and, consequently, oxidative stress-related events (for review see (Smith, M. A. et al., 1995)).

Hyperglycemia is known to differently affect different brain regions being that the cerebral cortex is highly sensitive to glucose fluctuations (Serpa, Jesus et al. 2006, Cardoso, Santos et al. 2010). Indeed, it has been reported that cortical neurons and astrocytes are more vulnerable to glucose metabolism deregulation than cells from striatum or hippocampus (Xu, L. et al., 2001). Moreover, it has also been reported that DM increases the vulnerability of specific brain areas to neuronal damage being the cerebral cortex particularly sensitive (Bree, A. J. et al., 2009). Furthermore, the cerebral tissues such as the cortex are quite vulnerable to OS due to its high consumption of oxygen, the abundance of easily oxidizable fatty acids (for review see (Wang, X. and Michaelis, E. K. 2010)), and the relative low presence of antioxidant defenses in comparison with other tissues. For example, the brain has 10% less antioxidant defenses than the liver (for review see (Uttara, B. et al., 2009)). Moreover, brain has higher levels of iron in certain regions and in general has high levels of ascorbate. Therefore, neural cells are considered to be more susceptible to oxidative damage as compared to other body tissues (Floyd, R. A. and Carney, J. M. 1992).

DM besides increasing the probability of occurrence of a stroke (Baird, T. A. et al., 2002), also increases the risk of cognitive impairments and dementia (for review see (Biessels, G. J. and Gispen, W. H. 2005)). Furthermore, it is becoming evident that diabetics have a higher risk for developing neurodegenerative diseases. Cerebral cortex is greatly affected by Alzheimer's disease (AD), and several studies have demonstrated that AD and DM are connected (for review see (Moreira, P. I. 2012)). Insulin resistance and its signaling impairment, mitochondrial abnormalities, OS, are some of the relevant events in both disorders (Santos, R. X. et al., 2014b). Therefore, hyperglycemia and other DM-related alterations have several negative effects on brain function and structure.

2.1 Hyperglycemia and Brain Metabolism

The brain depends on glucose as its main source of energy. In the adult brain, neurons have the highest energy demand (Howarth, C. et al., 2012), requiring continuous delivery of glucose from blood. This organ is metabolically very active, consuming 20% of the total body's oxygen and receiving 15% of the cardiac output (for review see (McCall, A. L. 2004)), in resting state. Energy consumption by the brain is largely needed to maintain and restore ionic gradients associated with synaptic transmission (for review see (McCall, A. L. 2004)), and the chemical energy within the brain exists primarily in the form of high-energy phosphate bonds contained in creatine phosphate and adenosine triphosphate (ATP).

The largest proportion of energy in the brain is used for neuronal computation and information processing; for example, the generation of action potentials and postsynaptic potentials generated after synaptic events and the maintenance of ion gradients and neuronal resting potential (Howarth, C. et al., 2012). Moreover, glucose metabolism provides the energy and precursors for the biosynthesis of neurotransmitters (for review see (Dienel, G. A. 2012)).

Dependence of the brain on glucose as its obligatory fuel derives mainly from the blood-brain barrier (BBB) and its selective permeability for glucose. Glucose cannot be replaced as an energy source, but it can be supplemented, as during strenuous physical activity when blood lactate levels are elevated (van Hall, G. et al., 2009) or during prolonged starvation (Lutas, A. and Yellen, G. 2013) when blood levels of ketone bodies are elevated. Cerebral metabolism of glucose requires transport through the BBB, glycolytic conversion to pyruvate, metabolism via the tricarboxylic acid (TCA) cycle and ultimately oxidation to carbon dioxide and water for full provision of ATP and its high-energy equivalents. Any disturbance in glucose metabolism compromises the brain normal functioning.

Brain is compromised by metabolic changes associated to DM, leading to cognitive deficits and to an increased risk of brain vascular complications (for review see (Biessels, G. J. and Gispen, W. H. 2005)). Thus, it is expectable that glucose metabolism dysfunction promoted by DM, particularly T2DM, is responsible for severe brain damage (for review see (McCall, A. L. 2004)). High blood glucose levels, obesity, increased blood triacylglycerol's concentration and insulin resistance, are some risk factors that, individually or collectively, increase the probability of neurodegeneration or even neuronal death (Duarte, A. I. et al., 2013).

Hyperglycemia has a variety of adverse effects upon brain metabolism and function (Diaz-Parejo, P. et al., 2003, Alves, M. G. et al., 2012). Glucose is a primary fuel for the brain, but lactate also plays an important role (Pellerin, L. et al., 2002). Animal studies have shown that there is a reduced overall glucose metabolism and regional changes in glucose metabolism in individuals with poorly controlled DM (Jakobsen, J. et al., 1990). The brain glucose metabolism is crucial to normal cerebral functioning. Under normal conditions, glucose crosses BBB through specific glucose transporters (GLUTs), glucose transporter 1 (GLUT1) and glucose transporter 3 (GLUT3). The rate of entry of glucose into the cell is limited by the number of GLUTs on the cell surface and the affinity of the transporters for glucose. Glucose is phosphorylated by hexokinase (HK) to produce glucose-6-phosphate that is then converted to pyruvate by phosphofructokinase-1 (PFK-1). The reaction catalyzed by this enzyme is usually described as one of the most important regulatory steps in glycolysis. PFK-1 activity is essential since it is responsible for the

conversion of fructose 6-phosphate to fructose 1,6-bisphosphate, the first irreversible step of glycolysis, and a limiting step of glycolytic flux (Underwood, A. H. and Newsholme, E. A. 1965). The pyruvate can be processed into three main metabolic pathways: a) transported into the mitochondrial matrix to form acetyl coenzyme A (acetyl-CoA); b) converted to lactate by lactate dehydrogenase (LDH); or converted to alanine by alanine aminotransferase (ALT). The lactate produced can be released in the extracellular space through monocarboxylate transporters (MCTs), mainly monocarboxylate transporter 4 (MCT4) (for review see (Oliveira, P. F. et al., 2014)).

The deleterious effects of hyperglycemia are mediated through an increased flux of glucose through the polyol and hexosamine pathways, disturbances of intracellular second messenger pathways, an imbalance in the generation and scavengers of ROS, and by AGEs (for review see (Brownlee, M. 2001)).

3. Brain Oxidative Stress and Hyperglycemia

OS may be defined as a measure of the steady-state level of reactive oxygen or oxygen radicals in biological systems. DM has been linked to ROS since the early 90s (for review see (Baynes, J. W. 1991)). OS is present in the early (prediabetes state) and late phase of DM (Su, Y. et al., 2008). Nowadays, it is widely accepted that OS is important in the development and progression of DM (for review see (Ceriello, A. 2000)). It has been reported that DM-related hyperglycemia and the glycemia fluctuations can amplify OS (for review see (Brownlee, M. 2001)) by increasing the production of free radicals and/or by impairing antioxidant defenses (for review see (Bloch-Damti, A. and Bashan, N. 2005)).

Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death. Various mechanisms have been suggested to contribute to the formation of ROS. Glucose oxidation, non-enzymatic glycation of proteins, oxidative degradation of glycated proteins and the mitochondrial respiratory system form free radicals in diabetic individuals (for review see (Maritim, A. C. et al., 2003)). Nevertheless, moderate amounts of ROS are important for various biological processes (for review see (Juranek, I. et al., 2013)).

OS occurs when there is an overproduction of ROS and/or a decreased efficiency of radical scavengers, such as glutathione (Bravi, M. C. et al., 2006). Glutathione is one of the most important intracellular antioxidants (for review see (Niedowicz, D. M. and Daleke, D. L. 2005)) and the relative amount of intracellular reduced and oxidized glutathione (GSSH) is a measure of the cellular redox status. Experimental and clinical studies have shown that glutathione levels are altered in diabetic patients (Dinçer, Y. et al., 2002).

It has been shown that ROS are produced in various tissues under diabetic conditions (for review see (Baynes, J. W. and Thorpe, S. R. 1999)), including the brain tissue. The hyperglycemia-related increase in ROS and reactive nitrogen species (RNS) can be due to several factors, such as mitochondrial respiratory system (for review see (Nishikawa, T. and Araki, E. 2007)), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (for review see (Gao, L. and Mann, G. E. 2009)), formation of AGEs (for review see (Brownlee, M. 2001)), and imbalance of glutathione redox status (Bravi, M. C. et al., 2006). As discussed above, under normal conditions, glucose enters the glycolytic pathway, through GLUTs, it is metabolized to pyruvate that is transported to the mitochondrial matrix, oxidized and decarboxylated by the pyruvate dehydrogenase forming the two carbon intermediate acetyl-CoA, which can enter the TCA cycle. However, the mitochondrial electron transport chain (ETC) is the main ROS producer (mainly the mitochondrial complex I and III) (for review see (Martin, S. D. and McGee, S. L. 2014)), and abnormal levels of blood glucose leads to production of larger amounts of ROS, leading to

OS and contributing to peripheral insulin resistance (Figure 1). Higher levels of blood glucose lead the increased supply of energy substrates and the inflammatory environment is thought to result in excessive mitochondrial ROS generation (Loh, K. et al., 2009). Thus, is possible that certain signaling pathways are activated and consequently induce insulin resistance (for review see (Kim, J. A. et al., 2008, Tiganis, T. 2011)). Increased ROS production associated with a reduction in plasma antioxidants, particularly glutathione, may have toxic effects on the plasma membrane structure/activity of the pancreatic beta-cells, contributing to the impaired insulin secretion (for review see (Paolisso, G. and Giugliano, D. 1996)). Furthermore, direct evidence for the involvement of hyperglycemia-induced ROS in promoting insulin resistance has been provided using mouse and rat animal models (Haber, C. A. et al., 2003, Anderson, E. J. et al., 2009). However, is important to note that ROS might be both good and bad, promoting insulin sensitivity early in disease progression, and contributing to the development of insulin resistance later, when hyperglycemia prevails (for review see (Tiganis, T. 2011)). On the other hand, sustained activation of NADPH oxidase leads to impaired antioxidant defenses due to superoxide production (for review see (Gao and Mann 2009)). If the level of NADPH decreases, the recycling of reduced glutathione (GSH) is limited (glutathione reductase uses NADPH to regenerate GSH). Particularly, increased NADPH oxidase activity contributes to a large number of pathologies such as DM, cardiovascular diseases and neurodegeneration. Noteworthy, it has been proposed that sustained activation of NADPH oxidase in DM leads to ROS production and impaired antioxidant defenses (for review see (Gao and Mann 2009)).

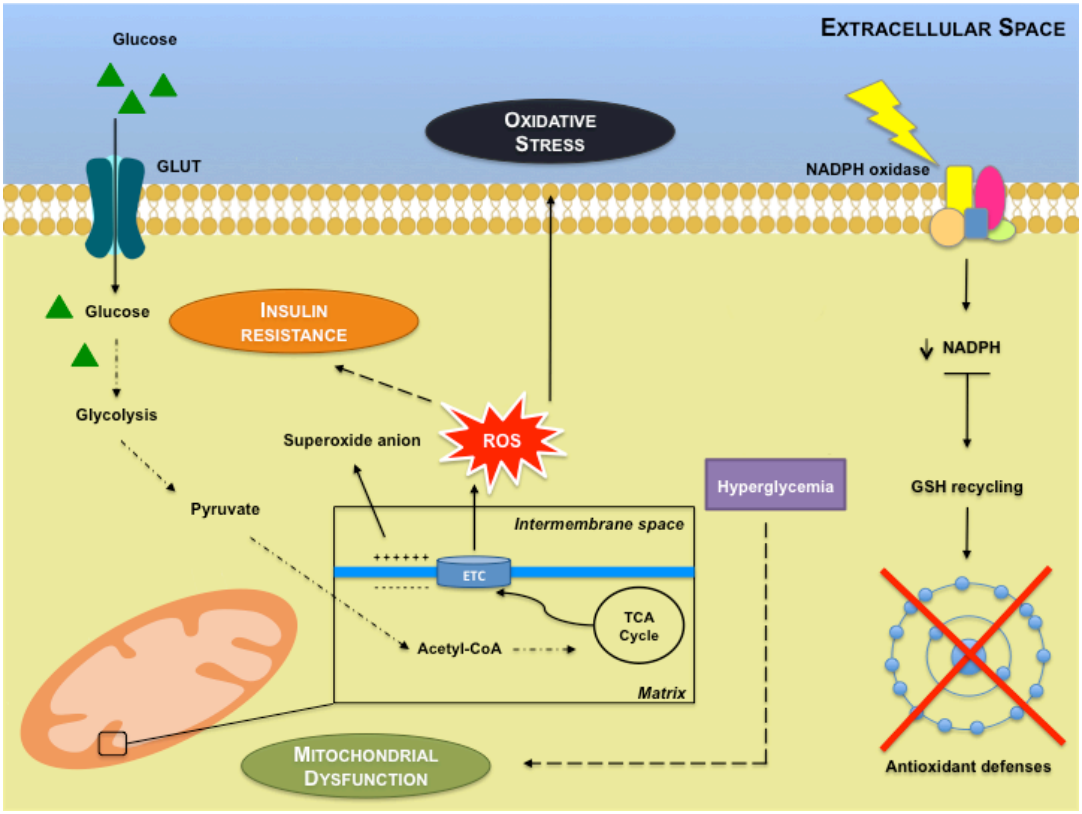


Figure 1 - Mitochondrial dysfunction and sustained activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase lead to insulin resistance, reactive oxygen species (ROS) production and impaired antioxidant defenses. Mitochondria are the main generators of ROS within electron transport chain (ETC). In normal conditions, glucose breakdown starts by glycolysis, generating among other compounds, pyruvate. Pyruvate is then converted to acetyl coenzyme A (acetyl-CoA) that enters the tricarboxylic acid (TCA) cycle. The produced electrons are stored in molecules that are then injected into the ETC, to generate the electrochemical gradient. When an abnormal increase in the electrochemical potential difference in the inner membrane of the mitochondria occurs leads to the overproduction of $O_2^{\cdot}$. This is particularly important since ROS may contribute to insulin resistance. Sustained activation of NADPH oxidase leads to decreased intracellular levels of NADPH and therefore the recycling of reduced glutathione (GSH) is limited, impairing antioxidant defenses.

Lipid peroxidation and protein carbonyls are biomarkers of OS. The abnormal enhancement of free radicals and the decline of antioxidant defense mechanisms lead to the damage of cellular organelles and enzymes, the increase in lipids peroxidation and the increase of insulin resistance (for review see (Maritim, A. C. et al., 2003)). The nonradical oxidants such as hydrogen peroxide, hypochlorous acid, singlet oxygen and radicals like superoxide anion and hydroxyl anion, can attack the double bound of unsaturated fatty acids promoting the formation of lipid peroxides (for review see (Lipinski, B. 2001)). On the other hand, protein oxidation originates carbonyl groups and their level in tissues and plasma is a stable marker of OS (Odetti, P. et al., 1999).

New ways to reduce the brain damage caused by DM may arise by modifying lifestyles, particularly by changes in diet. There is a large interest in finding an effective

therapy for DM-associated brain dysfunction and white tea seems to be a good candidate, with interesting properties such as antidiabetic (Dieren, S. v. et al., 2009), neuroprotective (Unno, K. et al., 2007, López, V. and Calvo, M. I. 2011) and antioxidant (Almajano, M. P. et al., 2008) properties. Thus, tea and its phytochemicals properties may be important and will be discussed below.

4. Tea

Since ancient times, medicinal plants have been used to prevent and treat a wide range of diseases. *Camellia sinensis* (L.), commonly known as the tea plant, is an evergreen shrub of the Theaceae family, native to Southeast China and is now cultivated in over 30 countries across the world (López, V. and Calvo, M. I. 2011), including S. Miguel Island (Azores Archipelago, Portugal).

Tea is one of the most widely consumed beverages in the world, surpassed only by the water (Cheng, T. O. 2006), with a per capita consumption of approximately 120 mL/day (Mckay, D. L. and Blumberg, J. B. 2002). The popularity of tea consumption is probably related with its sensorial properties, relatively low retail price, stimulating effects and potential health benefits (Moderno, P. M. et al., 2009, Dias, T. R. et al., 2013, Dias, T. R. et al., 2014, Martins, A. D. et al., 2014).

The origins of tea are mythological. The "Father of Tea", Eisai, said: "Tea is a miraculous medicine for the maintenance of health. Tea has an extraordinary power to prolong life." (Wheeler, D. and Wheeler, W. 2004). In fact, tea has been extensively used by traditional Chinese medicine for centuries to prevent and treat several diseases, such as DM (Wheeler, D. and Wheeler, W. 2004, Moderno, P. M. et al., 2009).

4.1 Types of Tea

Tea is an infusion prepared from the leaves of *C. sinensis*, but each type of tea has a different composition, which depends on the type of processing, growing conditions, botanical variety, and geographical origin (for review see (de Mejia, E. G. et al., 2009)). According to processing and collection, tea can be classified into black tea (completely fermented), oolong tea (semi-fermented), green tea and white tea (not fermented). After collection, the leaves gain a darker color, which means that chlorophylls are breaking down and tannins are being released. Upon harvesting, the leaves suffer oxidation, commonly called "fermentation", which occurs with the exposure to air and is a reaction catalyzed by the enzyme polyphenol oxidase (PO) (for review see (Mckay, D. L. and Blumberg, J. B. 2002)). Depending upon the level of "fermentation", all types of tea have different chemical compositions (phenolic profiles) and organoleptic properties (appearances and tastes).

To produce green tea, the leaves are rolled and steamed to minimize the oxidation by inactivation of the PO before drying (for review see (Mckay, D. L. and Blumberg, J. B. 2002)). Thus, the chemical composition of green tea remains similar to that of the *C. sinensis* fresh leaves. In black tea, the most consumed type of tea in the western countries (Li, S. et al., 2013), the leaves are rolled and cellular compartmentalization is disrupted bringing the phenolic compounds to contact with PO and then they undergo oxidation for 90 to 120 minutes (Rusak, G. et al., 2008). Oolong tea is produced with a shorter oxidation period than black tea and has a taste and color somewhere between green tea and black tea (Rio, D. D. et al., 2004) (Figure 2). Finally, white tea is the rarest and most expensive tea, and how it is produced and its chemical composition will be discussed below.

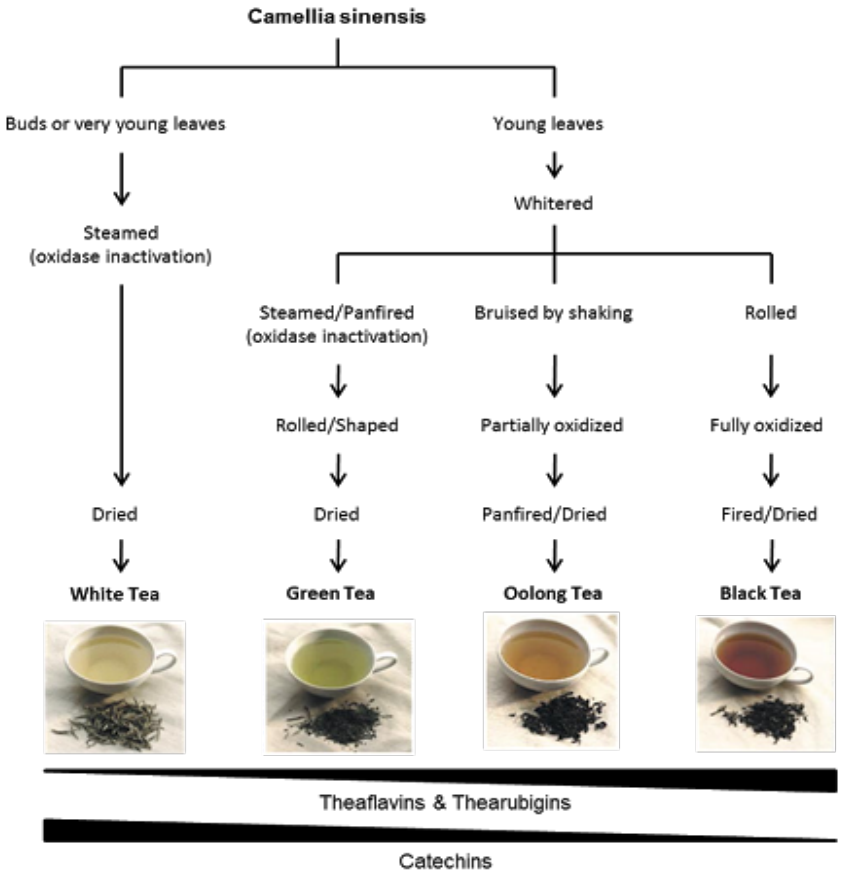


Figure 2 - Schematic representation of tea processing (adapted from (Dias, T. R. et al., 2013)).

All four types of tea are significant sources of antioxidant (Costa, R. M. et al., 2009, Moderno, P. M. et al., 2009), antidiabetic (Song, E. K. et al., 2003, Abolfathi, A. A. et al., 2012) and neuroprotective (Unno, K. et al., 2007, López, V. and Calvo, M. I. 2011) compounds.

4.2 White Tea

White tea is prepared from very young tea leaves or buds covered with tiny, silvery hair, which are harvested only once a year in the early spring (Rusak, G. et al., 2008). To prevent oxidation, white tea is steamed and dried immediately after harvest. The buds may be shielded from sunlight during growth to reduce the formation of chlorophylls, giving the young leaves a white appearance (Alcázar, A. et al., 2007). It is one of the less studies teas but its flavor is more accepted in Europe than that of green tea (Almajano, M. P. et al., 2008).

Many health benefits have been attributed to tea consumption. However, scientific investigations of this beverage and its constituents have been underway for less than three decades. In spite of numerous data about the phenolic constituents, antioxidant activity and ameliorating effects of green and black tea on human health, little is known in this sense about white tea, which is the rarest and the least processed tea (Rusak, G. et al., 2008). The possible beneficial health effects of white tea are being extensively investigated and have received a great deal of attention in recent years by our research group.

4.2.1 Chemical Composition

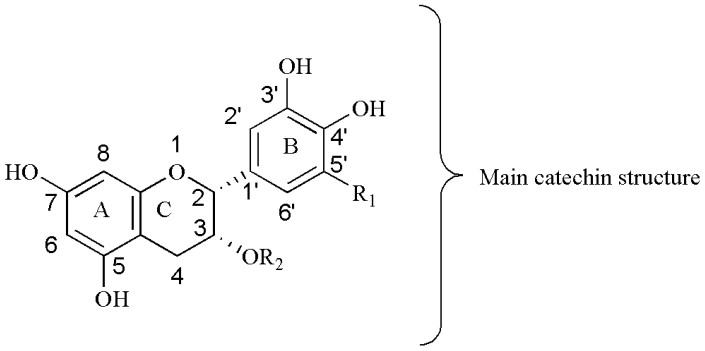
Tea is composed by a complex mixture of about 2000 chemical compounds, including proteins, polysaccharides, minerals and trace elements, organic acids, lignins, polyphenols, methylxanthines and amino acids (Seeram, N. P. et al., 2006, Moderno, P. M. et al., 2009). Several of these compounds are bioactive and are believed to possess health benefits (Carvalho, M. et al., 2010, Dias, T. R. et al., 2014, Martins, A. D. et al., 2014). Phenolic compounds, methylxanthines (mainly caffeine) and L-theanine have received particular attention among tea phytochemicals.

4.2.1.1 Phenolic compounds

Polyphenols are secondary plant metabolites, widely distributed in nature, and are the most abundant and active group of compounds present in tea. Catechins (also known as flavan-3-ols) and their derivatives are the main class of phenolic compounds present in tea leaves, constituting about 30% of their dry weight. The major catechins are (-)-epicatechin (EC), (-)-epigallocatechin (EGC) collectively known as flavanol monomers, (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin-3-gallate (EGCG) which are also called flavanol gallates (de Mejia, E. G. et al., 2009, Dias, T. R. et al., 2014). The health benefits attributed to catechins are mainly due to its chemical structure. The main catechins are

composed by two aromatic rings (A and B) linked to a dihydropyran heterocyclic ring (C) and are characterized by the presence of several hydroxyl groups (for review see (Braicu, C. et al., 2013)) (Figure 3). Their chemical differences are due to the presence of different groups attached to those rings. In EC, we can find an ortho-di-hydroxil group in the B ring (at carbons 3' and 4') and a hydroxyl group in the C ring (at carbon 3); EGC, which is an ester derivative of EC, additionally contains a gallate moiety esterified in the C ring, at carbon 3. EGC, on the other hand, possesses a trihydroxil group on the B ring (at carbons 3', 4' and 5'), and EGCG differs in this structure by additionally possessing an esterified gallate at the carbon 3 of the C ring. Green and white teas are the types of tea with higher catechin content, while oolong and black tea possess other phenolic compounds, in addition to lower catechins levels (for review see (Lin, Y. S. et al., 2003, Dias, T. R. et al., 2013, Li, S. et al., 2013)). Tea composition is affected by the oxidation process, a reaction catalyzed by PO that is released during the crushing of the leaves in the production of black and oolong tea, and catalyzes the oxidation and polymerization of the catechins EC, ECG, EGC and EGCG, producing theaflavins and thearubigins (for review see (Lin, Y. S. et al., 2003, Li, S. et al., 2013)). These oligomers/polymers are responsible for black tea bitter taste and dark color (Wheeler, D. and Wheeler, W. 2004). Theaflavins possess a basic chemical skeleton comprised of the bicyclic benzotropolone ring and are the result of main catechins dimerization (for review (Li, S. et al., 2013)). Thearubigins are produced subsequently to a series of complex reactions that form its oligo-polymeric structures.

EGCG is the most abundant catechin in tea leaves and has been extensively studied by several authors (for review see (Yang, C. S. et al., 2004, Seeram, N. P. et al., 2006)). It represents 50–80% of the total catechins, and is thought to contribute to the beneficial effects ascribed to tea (for review see (Khan, N. and Mukhtar, H. 2007)).



	R1	R2
(-)-epicatechin	H	H
(-)-epigallocatechin	OH	H
(-)-epicatechin-3-gallate	H	Gallate group
(-)-epigallocatechin-3-gallate	OH	Gallate group

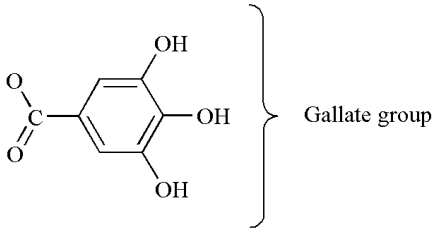


Figure 3 - Chemical structures of the main tea catechins. The figure illustrates two aromatic rings (A, B) and a dihydropyran heterocyclic ring (C), which is the basic structure of flavonoids. The (-)-epicatechin (EC) is constituted by an ortho-di-hydroxil group in the B ring (at carbons 3' and 4') and a hydroxyl group in the C ring (at carbon 3), and its ester derivative (-)-epicatechin 3-gallate (ECG) differs in this structure by possessing an additional gallate moiety esterified in the C ring, at carbon 3. On the other hand, (-)-epigallocatechin (EGC) contains a trihydroxil group on the B ring (at carbons 3', 4' and 5') and its ester derivative (-)-epigallocatechin-3-gallate (EGCG) additionally possesses an esterified gallate at the carbon 3 of the C ring.

The redox properties of phenolic compounds are in the basis of the tea antioxidant properties, which can be very useful if its consumption is adopted as a natural health practice (Atoui, A. K. et al., 2005). Several reports have shown that tea catechins and other polyphenols are effective scavengers of ROS and RNS (Guo, Q. et al., 1999, Paquay, J. B. et al., 2000). This is of extreme relevance since OS is known to induce neuronal death and to be involved in neurodegenerative diseases (for review see (Agostinho, P. et al., 2010, Dumont, M. et al., 2010). Thus, there is a growing interest in the possible neuronal tea benefits for DM patients.

4.2.1.2 Methylxanthines

Methylxanthines are purine bases derivatives present in tea, 2-4% as caffeine and small amounts of theophylline and theobromine (Hara, Y. et al., 1995). Caffeine (1,3,7-trimethylxanthine) is one of the most consumed substances in the world (Hashimoto, T. et al., 2004) and due to its chemical stability, the oxidation process does not affect caffeine levels in tea (for review see (Li, S. et al., 2013)). However, some researchers found that black and oolong tea have greater caffeine content than green tea (Lin, Y. S. et al., 2003) and that white tea has also a higher content than green tea (Unachukwu, U. J. et al., 2010, Dias, T. R. et al., 2014). These discrepancies may be due to different extraction conditions (solvents, temperatures, times of extraction and ratio leaves/water) and of distinct analytical methods. Besides, the natural variability of plants caused by edapho-climatic factors, harvesting techniques or agricultural practices may contribute to these differences.

The excessive consumption of caffeine can cause many adverse effects (for review see (Nawrot, P. et al., 2003)), such as nervousness, irritability, insomnia, diuresis, arrhythmia, tachycardia and gastrointestinal disturbances. Death provoked by excessive intake of caffeine, although rare, has also been reported (for review see (Nawrot, P. et al., 2003)). Some authors argue that the lowest caffeine content in green tea contributes to its beneficial health properties, mainly attributed to its phenolic compounds (Lee, L.-S. et al., 2013). Nevertheless, there are also some studies that highlight the potentially beneficial action of caffeine. Similarly to tea catechins, caffeine also has different effects at cellular and metabolic levels (for review see (Mandel, H. G. 2002)). The most important mechanism by which caffeine can act in Central Nervous System (CNS) is by selectively blocking the adenosine receptors and competitively inhibiting the action of adenosine in the cells, which results in an increased release of hormones such as norepinephrine, dopamine and serotonin (for review see (Nawrot, P. et al., 2003)). Caffeine is also a likely candidate against memory loss (for review see (Cunha, R. A. 2008)) and with a great neuroprotective potential (Cunha, R. A. 2005, Duarte, J. M. et al., 2009). Accordingly, studies in rats have shown that this methylxanthine can interact with GLUTs in adipocytes and act as an antagonist of adenosine receptors (Steinfelder, H. J. and Pethö-Schramm, S. 1990). Also, intravenous administration of caffeine to healthy human subjects resulted in decreased whole-body glucose uptake along with a decrease in carbohydrate storage (Greer, F. et al., 2001), as well as an increase in insulin insensitivity, resulting from the caffeine-induced release of the insulin-antagonistic hormone epinephrine (Keijzers, G. B. et al., 2002). Increases in blood pressure have also been reported (Keijzers, G. B. et al., 2002). Interestingly, the consumption of caffeine-containing beverages, in particular tea, is associated with a lower risk of developing T2DM (Dieren, S. v. et al., 2009, Sartorelli, D. S. et al., 2010). Some authors have also reported that caffeine intake is inversely associated

with body weight increase and satiety (Westerterp-Plantenga, M. S. et al., 2005, Lopez-Garcia, E. et al., 2006). Caffeine and theophylline are also involved in the stimulation of pancreatic beta cells (Johnston, K. L. et al., 2003). In the brain it has been shown that increased levels of caffeine are associated with decreased risk of neurodegenerative diseases (Chen, J.-F. et al., 2001). However, data on the role of caffeine on tea-associated health benefits are scarce and much work needs to be done.

4.2.1.3 L-theanine

L-theanine is a free amino acid which presents structural similarity to glutamate, an important neurotransmitter related to memory (for review see (Kakuda, T. 2011)). L-theanine constitutes between 1 and 3% of the dry weight of tea, but this percentage may vary according to growing location and method of cultivation, tea grade, variety, processing and collection time (Vuong, Q. V. et al., 2011). Green tea contains lower or similar levels of L-theanine as compared to black and oolong tea (Ekborg-Ott, K. H. et al., 1997). This amino acid is considered as a relaxing agent with antioxidant (Nishida, K. et al., 2008, Patti, M. E. and Corvera, S. 2010) and neuroprotective effects (Egashira, N. et al., 2007, Cho, H. S. et al., 2008, Kakuda, T. 2011). However, its pharmacology is relatively unknown and human studies are inconclusive (Lu, K. et al., 2004). Metabolically, it is easily absorbed from the gastrointestinal tract and peak plasma concentrations are detected 0.5 hour after administration (Kakuda, T. 2011). According to Yokogoshi and collaborators (1998), L-theanine is partially transported to the brain via a leucine-preferring transporter system and can cross the BBB, exercising protector effects in the brain and a preventive effect on neuronal cell death. The benefits of L-theanine for health are reported to be associated with regulation of blood pressure, effective prophylaxis and treatment of neurodegenerative diseases, improvement of the immune system, among others (Yokogoshi, H. and Kobayashi, M. 1998, Rogers, P. J. et al., 2008, Di, X. et al., 2010, Takagi, Y. et al., 2010).

5. White Tea Potential and Health Benefits

Bioactive components of plants have served as sources of inspiration for generations of medicinal and organic chemists, and will continue to provide humankind with valuable agents of potential use in research, prevention, and treatment of several diseases, like DM. Medicinal plants used in pharmaceutical products to treat diabetic conditions have aroused considerable interest in recent years (for review see (Ayyanar, M. et al., 2008)), including white tea (Islam, M. S. 2011).

Conventionally, DM is treated with OADs in the case of T2DM, or with exogenous insulin in case of T1DM or T2DM uncontrolled on OADs. However, these drugs are not completely effective and have adverse effects. Natural compounds are considered to be less toxic and relatively cheaper than synthetic ones and large amounts can be consumed in everyday diet (for review see (Saxena, A. and Vikram, N. K. 2004)). Scientific papers concerning the health benefits of tea consumption are relatively recent, and the studies are not as conclusive as we could expect. Thus, the search for evaluating the efficacy and safety of tea, particularly white tea and its phytochemicals, has become one important area of research.

5.1 Antioxidant Potential

In the last few years, antioxidant components have aroused great interest because of their ability to scavenge free radicals, reducing the harmful effects of ROS and RNS, thereby inhibiting oxidation (Alarcón, E. et al., 2008). The majority of living organisms possess efficient enzymatic and nonenzymatic defense systems against excessive production of ROS. Nevertheless, factors such as lifestyle (smoke, diet, alcohol, some drugs, among others) and internal factors (such as aging) decrease the efficiency of endogenous antioxidant defenses, creating an impairment in the redox equilibrium that is established in healthy conditions (for review see (Rietveld, A. and Wiseman, S. 2003)). Chronic exposure to ROS can damage DNA, membrane lipids, lipoproteins, and functional and structural proteins (Halliwell, B. 1997). Increased OS has been proposed to be one of the major causes of the hyperglycemia-induced diabetic complications (Valko, M. et al., 2007). Due to these events, the cellular balance between radical formation and protection against them is disturbed. The elimination of ROS to decrease the oxidative damage is seen as beneficial to public health. Therefore, antioxidants that scavenge ROS may be of great value in preventing the onset and/or the progression of oxidative diseases (for review see (Willett, W. C. 1994)).

It is well known that hyperglycemia increases the formation of ROS and decreases antioxidant endogenous mechanisms (Rahimi, R. et al., 2005). Several studies have reported that tea phenolic compounds, mainly catechins, are potent antioxidant agents, scavenging ROS (Nakagawa, T. and Yokozawa, T. 2002) and metal chelators (Atoui, A. K. et al., 2005). Numerous studies have demonstrated that tea catechins and polyphenols are effective scavengers of physiologically relevant ROS and RNS *in vitro*, including superoxide (Nanjo, F. et al., 1993, Nakagawa, T. and Yokozawa, T. 2002), peroxyl radicals and singlet oxygen (Guo, Q. et al., 1999). The chemical structure of tea components is associated with its antioxidant properties. In this context, a relationship has been suggested between the content of pyrogallol and hydroxyl groups and the superoxide anion scavenging ability, as well as between the presence of galloyl moieties and the ability to quench hydroxyl radicals (Nanjo, F. et al., 1999, Moderno, P. M. et al., 2009). Several structures appear to be important for these antioxidant activities of tea polyphenols (TP), including the ortho-3',4'-dihydroxyl (catechol) group in the B-ring, that promotes the formation of a stable phenoxyl radical due to effective electron delocalization (Wiseman, S. A. et al., 1997) or the 3',4',5'-trihydroxyl (gallate) group in the B-ring, a gallate group esterified at the 3 position of the C-ring, and hydroxyl groups at the 5 and 7 positions of the A-ring (Rice-Evans, C. A. et al., 1996). It is known that the number and position of the hydroxyl groups on the molecules greatly influence the antioxidant ability of flavonoids (for review see (Braicu, C. et al., 2013)). Particularly, tea catechins such as EGCG lack a 2, 3 double bond and a carbonyl group at the 4-position, a combination that is known to strengthen the antioxidant activity (for review see (Moderno, P. M. et al., 2009). The structure of catechins has a major influence in their antioxidant properties, such as radical scavenging, transition-metal chelation, inhibition of redox sensitive transcription factors, inhibition of pro-oxidant enzymes and induction of antioxidant and phase II detoxification enzymes (for review see (Aboul-Enein, H. Y. et al., 2013, Braicu, C. et al., 2013)). Catechins antioxidant activities are related to their ability to enter in several chemical reactions of hydrogen atom and single electron transfers, involving hydroxyl groups. Studies have reported that the antioxidant properties of tea catechins are only observed *in vivo* when the animals are under OS, contrary to *in vitro* studies, where these activities can nearly always be observed. Studies have also demonstrated that catechins influence the levels of endogenous antioxidants. A study performed on rats by Srividhya and collaborators (2008) showed that continuous administration of EGCG for 30 days was able to significantly improve the animals' antioxidant defenses, ameliorating the age-induced OS in their brains. After analysis of the enzymatic and non-enzymatic antioxidants, lipid peroxidation and protein carbonyl groups on the animals brain tissue, the authors reported that EGCG successfully induced a rising in the activity of the antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase, as well as in the levels of non-enzymatic antioxidants such as L-ascorbic acid, α-tocopherol and glutathione. Also, lipid peroxidation and levels of protein

carbonyls (markers of protein damage induced by ROS) showed a significant decrease after EGCG treatment. Moreover, the authors verified that the treatment of young rats with EGCG, whose brain tissue did not display the oxidative stress levels induced by age, did not induce the same significant alterations in the antioxidant levels (Srividhya, R. et al., 2008).

Some authors state that white tea and green tea are the types of tea with greater catechins content and antioxidant properties. However, it should be noted that there is still some controversy in this matter. The measurement of antioxidant activity through several assays, denote that the higher total phenolic component may not always be correlated with greater antioxidant capacity, mainly because different phenolic profiles can yield different responses in the assays (Gorjanovic, S. et al., 2012). Among the catechins present in tea, EGCG is considered the most abundant and active (for review see (Moderno, P. M. et al., 2009, Dias, T. R. et al., 2013)), and its beneficial effects have been widely studied in different areas of research. The antioxidant power of catechins determined by the diphenylpicrylhydrazyl (DPPH) method was found to be: EGCG > ECG > EGC > EC (Katalinic, V. et al., 2006).

The knowledge of pharmacokinetics, absorption, distribution, metabolism, and excretion of tea components is essential to determine its potential bioactivities and overall significance in disease prevention (Wheeler, D. and Wheeler, W. 2004). Despite the proven antioxidant capacity of TP, many clinical studies and animal models have shown that these compounds, especially the polymers, esters, and glycosides, are abundant, but are not always absorbed by oral administration. The functional effect of the compound depends not only on the amount ingested, but on its bioavailability (Holst, B. and Williamson, G. 2008). Several clinical trials have demonstrated that a single dose of tea improves plasma antioxidant capacity of healthy adults within 30 to 60 minutes after ingestion (Benzie, I. et al., 1999). In a recent study, Koutelidakis and collaborators (2009) reported that supplementation of white tea extract for a five consecutive days not only increases the antioxidant capacity of plasma but also of different organs in mice, such as heart and lungs. Despite catechins, especially EGCG, theaflavins and flavonol glycosides are also thought to be responsible for antioxidant properties of tea. The antioxidant effectiveness depends on the tea variety and the content of EGCG is very important (Hilal, Y. and Engelhardt, U. 2007). Several epidemiological studies, experimentation with animals, and *in vitro* studies lead to the conclusion that white tea has potential for several protective effects for a wide variety of health problems.

5.2 Antidiabetic Potential

In recent years, interest has increased in using natural products for pharmacological purposes, as a form of complementary or replacement therapy, including to avoid the deleterious effects of DM. There are several reports showing that numerous extracts obtained from plants are efficient in reducing glycemia, causing fewer adverse effects and with lower costs than the usual OADs (Gupta, R. K. et al., 2005, Lee, M. S. and Sohn, C. B. 2009, Sohn, E. et al., 2010). There is some evidence that tea is an hypoglycemic agent (MacKenzie, T. et al., 2007). However, the exact mechanism by which tea ameliorates DM-related deleterious effects has not been elucidated yet. *In vitro* rat studies conducted by Anderson and Polansky (2002) suggest that EGCG and other catechins and theaflavins help to prevent hyperglycemia by enhancing insulin activity and possibly by preventing damage to pancreatic beta cells. The exact mechanisms by which TP ameliorate glycemic control are not clear but all studies suggest that polyphenols don't increase insulin secretion, but decrease insulin resistance and improve insulin sensitivity (Islam, M. S. 2011). An *in vivo* study in *db/db* mice, a model of obesity, DM and dyslipidemia, that received diets supplemented with EGCG for 10 weeks, showed improved glucose tolerance, increased glucose-stimulated insulin secretion and preservation of islets of Langerhans structure (Ortsäter, H. et al., 2012). Several studies with other types of tea that contain the same components of white tea showed a significance inverse association between the consumption and the risk of T2DM incident (Iso, H. et al., 2006, Khan, N. and Mukhtar, H. 2007, Odegaard, A. O. et al., 2008).

As previously discussed, prediabetes and DM are associated to metabolic changes, increased formation of ROS and RNS, and consequently increased OS and decreased antioxidant potential (for review see (Engelgau, M. M. et al., 2000, Rahimi, R. et al., 2005)). Increased OS has been proposed to be one of the major causes of the hyperglycemia-induced diabetic complications (for review see (Valko, M. et al., 2007)). Some studies showed that EGCG ameliorates cytokine-induced beta cell damage *in vitro* (Han, M. K. 2003) and prevents the decrease of islet mass induced by treatment with multiple low doses of streptozotocin (STZ) *in vivo* (Song, E. K. et al., 2003). Another study reported that supplementation with EGCG reduces serum glucose, total cholesterol and triglyceride and LDL-cholesterol in STZ-induced diabetic rats (Roghani, M. and Baluchnejadmojarad, T. 2010). This effect however can be different from the effect of the tea extract that contains several bioactive components. In contrast to studies indicating an antioxidant capacity of EGCG, investigations in the insulinoma cell line HIT-T15 showed that EGCG treatment was associated with increased production of ROS and reduced cell viability (Suh, K. S. et al., 2006). Thus, the antidiabetic effects of EGCG are not entirely clarified. The *in vivo* relevance of white tea catechins antidiabetic potential remains to be unravel. Recently, white tea was reported to have strong lipolytic and anti-adipogenic

activity *in vitro* (Sohle, J. et al., 2009). Hence, white tea consumption and/or its phytochemicals, namely EGCG, may demonstrate an antidiabetic effect by reducing hyperlipidemia and insulin resistance (Islam, M. S. 2011). Since hyperglycemia cases are increasing and are associated with several complications, there is a large interest in finding an effective therapy and white tea seems to be a good candidate.

5.3 Neuroprotective Potential

In the CNS, OS caused by increased production of ROS and RNS represents an important mechanism for neuronal dysfunction and cell loss in different neurodegenerative disorders (Almajano, M. P. et al., 2011). As discussed, the brain is particularly vulnerable to hyperglycemia-induced oxidative damage. Although neuronal cells may respond to OS using enzymatic and non-enzymatic detoxification mechanisms, there are certain populations of neurons that are particularly vulnerable to OS (for review see (Wang, X. and Michaelis, E. K. 2010)). Tea has been described to have a neuroprotective role due to its high polyphenolic content, mainly of catechins and other flavanols (Mandel, S. et al., 2006, Almajano, M. P. et al., 2008).

Isolated constituents from tea have previously been demonstrated to exert protective effects in neuronal cells. The neuroprotective properties of tea are greatly associated with EGCG. In fact, EGCG can easily cross the BBB and reach the brain parenchyma (Suganuma, M. et al., 1998). For instance, EGCG was shown to have neuroprotective activity in a mice model of Parkinson's disease (PD) (Levites, Y. et al., 2001), and an epidemiologic study indicated that the risk of PD was reduced if tea consumption (2 or more cups/day) occurred (Checkoway, H. et al., 2002). Moreover, long-term administration of green tea catechins showed to improve spatial cognition and learning ability in rats (Haque, A. M. et al., 2006), and to reduce cerebral amyloidosis in AD transgenic mice (Rezai-Zadeh, K. et al., 2005). A study by Rodrigues and collaborators (2013) evaluated the effects of catechins in an extract of green tea poor in EGCG. The authors showed that other catechins (EC, EGC and ECG) are effective protectors of proteins and lipids against oxidative changes related to aging. This study demonstrated that EGCG is not essential to some neuroprotective effects and that EC, EGC and ECG are able to improve behavioral performance and protect against oxidative damage. The potential neuroprotective effect of white tea extract on hydrogen peroxide-induced toxicity in PC12 cells has also been reported (López, V. and Calvo, M. I. 2011). These cells were treated with various doses of white tea (10–250 μg/ml) and cell survival was significantly increased in white tea-treated cells compared to hydrogen peroxide-treated cells. Tea demonstrated to have a good antioxidant power and was able to reduce the OS. Oral intake of tea catechins proved to be neuroprotective in animal models of neurotoxicity (Kang, K. S. et

al., 2010) and in animal models of aging, which are characterized by increased OS levels (Li, Q. et al., 2010).

On the other hand, caffeine is one of the components present in white tea, and has demonstrated a great neuroprotective potential (Cunha, R. A. 2005, Duarte, J. M. et al., 2009). In addition, L-theanine is ascribed to be a neuroprotective and cognitive enhancing agent (Nathan, P. J. et al., 2006, Dimpfel, W. et al., 2007). It can cross BBB to exert its effects directly on the brain within 30 minutes (Unno, T. et al., 1999). Recently, L-theanine has been linked to the feelings of relaxation reported by those who drink tea. Experimental studies have also shown that L-theanine appears to counteract some of the effects of caffeine (Kimura, K. et al., 2007). In this sense, several of the reports discussed herein suggest that protection afforded by tea consumption may be due to a synergistic effect among the various compounds of tea.

II. Aims of Project

DM represents one of the greatest threats to modern global health and its incidence is rapidly increasing. The prevalence of a prediabetic state, a prodromal stage of DM, is increasing among young people and is a major risk factor for the development of T2DM.

The brain, particularly the cerebral cortex, is very susceptible to fluctuations in glucose levels and hyperglycemia-induced OS. Besides, it also has low antioxidant defenses comparatively to other brain regions. It is also one of the most affected areas in neurodegenerative diseases.

Tea (*Camellia sinensis* (L.)) is widely consumed and several benefits, including antioxidant, antidiabetic and neuroprotective effects have been attributed to its consumption. However, there are few studies focused on white tea, the less processed tea type.

The aim of the present study was to evaluate the effects of the daily consumption of white tea in the cerebral cortex metabolic and oxidative profile of STZ-induced prediabetic rats.

III. Methods

1. Chemicals

All chemicals were purchased from Sigma-Aldrich (St.Louis, MO, USA), unless specifically stated otherwise.

2. White Tea Infusion

White tea samples were purchased on the Portuguese market, were originated from China and produced in European Union. Tea leaves were subjected to infusion (1 g per 100 mL of distilled water) at 100ºC, during 3 minutes, according to the manufacturer's instructions. The resulting infusion was filtered through a 0.2 µm cellulose acetate filter (VWR, Pennsylvania, USA). The phytochemical profile of white tea was previously determined by previous works from our team (Martins, A. D. et al., 2014).

3. Rats

Eighteen three-month-old male Wistar rats (*Rattus norvegicus*) were housed under a 12 hours light-12 hours darkness cycle and constant room temperature (20 ± 2 °C) in our accredited animal colony (Health Science Research Center, University of Beira Interior). Rats were maintained with *ad libitum* food and water. All animal experiments were performed according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and the European rules for the care and handling of laboratory animals (Directive 86/609/EEC).

4. Rat Model and Experimental Design

Prediabetes is a prodromal stage of DM characterized by elevated blood glucose levels, although not sufficient to meet the criteria for established diabetes (Association, A. D. 2010). Prediabetic individuals present IFG and/or IGT, and its prevalence is increasing among young people (Association, A. D. 2010). Prediabetes was induced by intraperitoneal (IP) administration of a low-dose of STZ, in accordance with the method described by Iwase and collaborators (1986), with slight modifications. In brief, two-days-old male Wistar rats from the prediabetes groups were injected with STZ (40 mg/kg, IP) freshly diluted in

citrate buffer (0.1 M sodium citrate, pH 4.5). The control group received only the vehicle solution in an equivalent volume. Rats' were fed *ad libitum* with a standard chow diet (4RF21 certificate, Mucedola, Italy).

At one month of age, STZ-treated rats were randomly divided in two groups. One group consumed white tea during 2 months (PrDM+WTea group). The other group of STZ-treated rats (PrDM group) and control rats (Control group) consumed water. Rats' weight and blood glucose levels were monitored every 6 days. Non-fasting glycemia was determined using a glucometer (One Touch Ultra Lifescan-Johnson, Milpitas, CA, USA). At the end of the treatment, rats were sacrificed by decapitation. Brain was removed and cerebral cortex tissue was collected, weighed and stored at -80ºC.

5. Insulin and Glucose Tolerance Test

At 3 months of age, rats were submitted to a glucose tolerance test (GTT), as previously described by Rato and collaborators (2013). In brief, 14-18 hours before the test, food was removed and rats kept in fast. An IP injection with 6 mL of glucose 30% (w/v) per kg of body weight was given to each rat. Blood samples for glucose measurement were obtained from the tail vein immediately before and 30, 60, 90 and 120 minutes after glucose administration. The rats were also subjected to an insulin tolerance test (ITT) as described by Rato and collaborators (2014). In brief, 16-18 hours before the test, food was removed and rats kept in fast. An IP injection with 0.75 U insulin per kg of body weight was administered to each rat. Blood samples for glucose measurement were obtained from the tail vein immediately before and 30, 60, 90 and 120 minutes after insulin administration. The areas under the curves for glucose tolerance (AUC_{GTT}) and insulin tolerance (AUC_{ITT}) tests were calculated using the trapezoidal rule as previously described (Rato, L. et al., 2014).

6. Ferric Reducing Antioxidant Power Assay

The ferric reducing antioxidant power (FRAP) of cerebral cortex was performed according to the colorimetric method described by Benzie and Strain (1996). In brief, cortex was homogenized in phosphate buffer solution (PBS) (pH 7.4). Protein concentration was determined by the Bradford micro-assay using bovine serum albumin (BSA) as standard. Antioxidant potential of the samples was determined against standards of L-ascorbic acid, by following the absorbance changes at 595 nm due to the reduction of the Fe3+-2,4,6-

Tripyridyl-s-Triazine (TPTZ) complex to a colored Fe2+-TPTZ complex by the samples. Absorbance results were corrected by using a blank, using H_2O instead of sample. The changes in absorbance values of tested reaction mixtures were used to calculate the FRAP value of the samples (µmol of antioxidant potential/mg protein).

7. Thiobarbituric Acid Reactive Species Assay

Thiobarbituric acid reactive species (TBARS) are formed as a byproduct of lipid peroxidation and can be detected by the TBARS assay using thiobarbituric acid (TBA) as a reagent. This peroxidation reaction produces malondialdehyde (MDA) that reacts with TBA in conditions of high temperature and low pH, generating a pink colored complex, which absorbs at 532 nm (Ohkawa, H. et al., 1979). TBARS assay was carried out by the method described by Iqbal and collaborators (1996) with slight modifications. In brief, 20 µg of tissue homogenate was mixed with Tris–HCl buffer (150 mM, pH 7.1), ferrous sulphate (1.0 mM), L-ascorbic acid (1.5 mM) and H_2O. This mixture was then incubated at 37°C for 15 minutes. The reaction was stopped by addition of trichloroacetic acid (10% w/v). Subsequently, TBA (0.375% w/v) was added and all samples incubated for 15 minutes at 100°C. Finally, samples were centrifuged at 1000×g for 10 minutes. The amount of MDA formed was estimated by measuring the optical density at 532 nm using an Anthos 2010 microplate reader (Biochrom, Berlin, Germany) against a blank. The results were expressed as nmol TBARS/mg tissue.

8. Analysis of Carbonyl Groups

Protein carbonyl content is commonly used as a marker for protein oxidation. Evaluation of the protein carbonyl groups content was performed using the slot-blot technique. First, samples were derivatized using 2,4-dinitrophenylhydrazine according to the method developed by Levine and collaborators (1990). Derivatized samples were then diluted in PBS and transferred to activated polyvinylidenedifluoride membranes by the slot-blot technique, which was performed using a Hybri-slot manifold system (Biometra, Göttingen, Germany). The membranes were then blocked by incubating during 90 minutes with 5% non-fat milk TBS solution with 0.05% Tween-20. Afterwards, membranes were incubated overnight with rabbit anti-2,4-Dinitrophenol (DNP) antibody (1:5000, Sigma Aldrich, Roedermark, Germany, D9656) and then incubated with an anti-rabbit alkaline phosphatase linked immunoglobulin G (IgG-AP) (1:5000, Santa Cruz Biotechnology

Heidelberg, Germany, SC-2007). Membranes were then reacted with enhanced chemifluorescence (ECF) substrate (GE, Healthcare) and read using a BioRad FX-Pro-plus (Bio-Rad, UK). Densities from each band were quantified using the BIO-PROFIL Bio-1D Software from Quantity One (VilberLourmat, Marne-la-Vallée, France).

9. Gluthatione Assay

Total glutathione and GSH levels in cerebral cortex were evaluated using a method developed by Baker and collaborators (1990) with slight modifications. In brief, 50 mg of cortex were homogenized in 5% 5-sulfosalicylic acid (SSA) and centrifuged at 10000xg for 10 minutes. The supernatant was then collected, diluted 5-fold and used for the determination of total glutathione. To evaluate GSH levels, samples were first derivatized with 2-vinylpyridine. This was done by incubating each sample in a 2-vinylpyridine solution (1 M) for 60 minutes. Glutathione levels were measured using a kinetic essay in which glutathione causes a continuous reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to 2-nitro-5-mercaptobenzoic acid (TNB), which can be spectrophotometrically measured at 412 nm. Standards with known concentrations (50, 25, 12.5, 6.25 and 3.125 μM of GSH) were prepared and a standard curve was done. The levels of GSSH were calculated by subtraction of the results obtained for total glutathione and GSH. The results were expressed as nmol of glutathione per mg of tissue.

10. Western Blot

Total proteins were isolated from cerebral cortex using RIPA lysis buffer (1x PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride (PMSF), supplemented with 1% protease inhibitor cocktail and 100 mM sodium orthovanadate). Western blot was performed as previously described (Alves, M. G. et al., 2011). Membranes were incubated with rabbit anti-GLUT1 (1:200, Millipore, Temecula, USA, CBL242), or rabbit anti-GLUT3 (1:250, Santa Cruz Biotechnology, Heidelberg, Germany, SC-31838), or rabbit anti-PFK-1 (1:1000, Santa Cruz Biotechnology, Heidelberg, Germany, SC-67028), or rabbit anti-MCT4 (1:1000, Santa Cruz Biotechnology, Heidelberg, Germany, SC-50329), or rabbit anti-LDH (1:5000, Abcam, Cambridge, USA, ab52488) or mouse anti-Catalase (1:4000, Sigma Aldrich, Roedermark, Germany, C0979). Mouse anti-α-tubulin was used as protein loading control (1:2500, Sigma Aldrich, Roedermark, Germany, T9026). The immune-reactive proteins were detected separately with goat anti-rabbit IgG-AP (1:5000, Santa

Cruz Biotechnology, Heidelberg, Germany, SC-2007) or goat anti-mouse IgG-AP (1:5000, Santa Cruz Biotechnology, Heidelberg, Germany, SC-2008). Membranes were reacted with ECF detection system (GE, Healthcare, Weßling, Germany) and read with the BioRad FX-Pro-plus (Bio-Rad, Hemel Hempstead, UK). The Quantity One Software (Bio-Rad, Hemel Hempstead, UK) was used to obtain band densities following standard procedures. The densities of each band were divided by the corresponding α-tubulin and expressed in fold variation versus the control group.

11. Lactate Dehydrogenase Activity Assay

The activity of LDH was spectrophotometrically assessed by determining the cleavage of a colorimetric substrate as described by Alves and collaborators (2011) using a commercial kit (Promega, Madison, WI, USA) and following the manufacturer's instructions. In brief, LDH activity was calculated by measuring the samples shift on the absorbance (492 nm) that resulted from the conversion of a tetrazolium salt (INT) into a red formazan product. The amount of formazan formed is directly proportional to the activity of LDH. The method was calibrated with LDH positive control included in the assay kit. The attained activities were calculated using the molar absorptivity of formazan and expressed as fold variation versus the control condition.

12. Proton Nuclear Magnetic Resonance

Intracellular metabolites of cerebral cortex samples were extracted using a methanol/chloroform/water strategy as previously described (Martineau, E. et al., 2011). The upper methanol-water phase containing the water-soluble cellular metabolites was carefully separated and lyophilized. For Proton Nuclear Magnetic Resonance (^{1}H-NMR) analysis, the lyophilized samples were dissolved in D_2O. ^{1}H-NMR spectra of the samples were acquired at 14.1 T, 25 °C, using a Bruker Avance 600 MHz spectrometer equipped with a 5-mm QXI probe with a z-gradient (Bruker Biospin, Karlsruhe, Germany) using standard methods (Alves, M. G. et al., 2011, Alves, M. G. et al., 2013b). Sodium fumarate was used as an internal reference (singlet, 6.50 ppm) to quantify the metabolites in solution (multiplet, d, ppm): valine (doublet, 1.02); aspartate (double doublet, 1.8); gamma-aminobutyric acid (GABA) (triplet, 2.28); glutamate (multiplet, 2.0); lactate (doublet, 1.33); alanine (doublet, 1.47); taurine (triplet, 3.4); N-acetylaspartate (NAA) (doublet, 7.9); fumarate (singlet, 6.5). The relative areas of ^{1}H-NMR resonances were quantified

using the curve-fitting routine supplied with the NUTSpro NMR spectral analysis program (Acorn NMR, Inc, Fremont, CA, USA).

13. Statistical Analysis

The statistical significance among the experimental groups was assessed by one-way ANOVA, followed by Bonferroni post-test. All experimental data are shown as mean ± SEM (n=6 for each condition). Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA). $P<0.05$ was considered significant.

IV. Results

1. Daily white tea consumption ameliorates glucose and insulin tolerance in prediabetic rats

At the end of the treatment, rats from the three experimental groups presented similar body weights (Table 1). STZ-treated rats developed typical characteristics of prediabetes. The average glycemia significantly increased from 90 ± 1 mg/dL in rats from the control group to 119 ± 2 mg/dL in rats from the prediabetic group (Table 1). These mild hyperglycemic values fit the diagnostic for the prodromal stage of T2DM, prediabetes.

Table 1 - Average values of the rats weight, blood glycemia, area under the curve for glucose tolerance (AUC_{GTT}) and insulin tolerance (AUC_{ITT}) tests in rats from the control, prediabetic rats drinking water (PrDM) and prediabetic rats drinking white tea (PrDM+WTea) groups after 60 days of treatment. Results are expressed as means ± SEM (n=6 for each condition). Significantly different results ($P < 0.05$) are indicated as: a - relative to control; b - relative to PrDM.

Parameters	Control	PrDM	PrDM+WTea
Weight (g)	347 ± 20	352 ± 32	378 ± 32
Glycemia (mg/dL)	90 ± 1	119 ± 2[a]	117 ± 2[a]
AUC_{GTT}	17661 ± 670	23364 ± 1095[a]	17760 ± 1446[b]
AUC_{ITT}	6870 ± 597	1592 ± 299[a]	4907 ± 871[b]

The rats were subjected to glucose tolerance and to insulin sensitivity tests and the area under the curve (AUC) was calculated in order to evaluate those parameters. STZ-treated rats developed glucose intolerance, as can be seen by the significantly higher AUC_{GTT} (23364 ± 1095 arbitrary units), when compared with the control rats (17661 ± 670 arbitrary units) (Table 1). The insulin resistance test further confirmed that STZ-treated rats developed typical characteristics of prediabetes, since they presented a significantly lower shift of the blood glycemia when subjected to an insulin resistance test, as represented by AUC_{ITT} (1592 ± 299 arbitrary units) as compared to the control rats (6870 ± 597 arbitrary units), illustrating a significant increase in insulin resistance (Table 1). White tea-consumption prediabetic rats, when subjected to an IP injection of glucose exhibited a

significantly smaller AUC_{GTT} value (17760 ± 1446 arbitrary units) than the prediabetic rats drinking water (Table 1). Furthermore, prediabetic rats drinking white tea also demonstrated a significantly lower insulin resistance (AUC_{ITT} of 4907 ± 871 arbitrary units) compared to water-drinking prediabetic rats (Table 1). Noteworthy, prediabetic rats that consumed white tea showed no significant differences in the GTT or in the ITT, when compared with the rats from the control group.

2. Daily white tea consumption decreases GLUTs expression and lactate accumulation in the cerebral cortex of prediabetic rats

We evaluated the expression of the two principal GLUTs (GLUT1 and GLUT3) in cerebral cortex. Our results show that prediabetes does not significantly alter GLUT1 protein levels in the cerebral cortex (Figure 4, Panel A). Nevertheless, white tea consumption significantly decreased GLUT1 protein levels in the cortex of prediabetic rats to 0.89 ± 0.06 fold variation of the control (Figure 4, Panel A). Moreover, white tea consumption also significantly decreases GLUT3 protein levels in the cortex of prediabetic rats (1.03 ± 0.05) to 0.82 ± 0.06 fold variation to the control (Figure 4, Panel B).

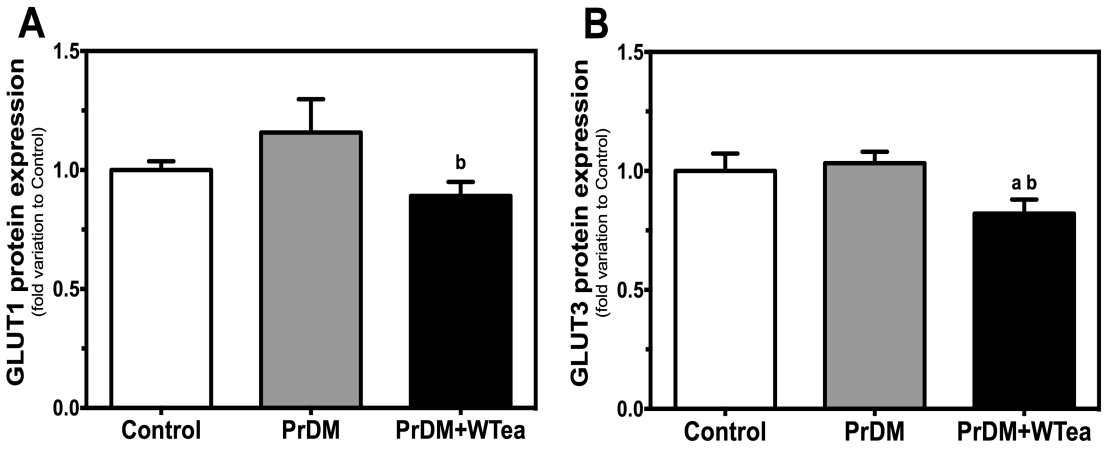


Figure 4 - Effect of daily white tea consumption in protein expression of glucose transporters (GLUTs) in the cerebral cortex of prediabetic rats. Protein expression of glucose transporter 1 (GLUT1) (Panel A) and glucose transporter 3 (GLUT3) (Panel B) in the cortex of control, prediabetic (PrDM) rats drinking water and PrDM drinking white tea (PrDM+WTea). Results are expressed as means ± SEM (n=6 for each condition). Significantly different results ($P < 0.05$) are indicated as: a - relative to control; b - relative to PrDM.

After glucose enters the brain, it follows the glycolytic pathway. PFK-1 is known rate-limiting step of this process. Our results show that PFK-1 levels in the cortex of prediabetic rats remained unchanged (Figure 5). Also, white tea consumption was unable to change PFK-1 protein expression levels in the cortex of prediabetic rats (Figure 5).

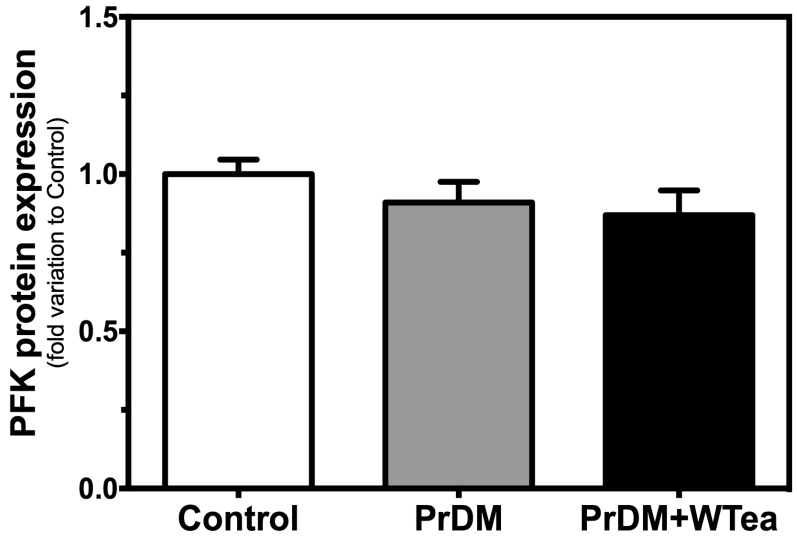


Figure 5 - Effect of daily white tea consumption in protein expression of phosphofructokinase-1 (PFK-1) in the cerebral cortex of prediabetic rats. Protein expression of PFK-1 in the cortex of control, prediabetic (PrDM) rats drinking water and PrDM drinking white tea (PrDM+WTea). Results are expressed as means ± SEM (n=6 for each condition).

One of the major contributors for neuronal lesions related to hyperglycemia is lactate overproduction. Our results show that LDH protein expression is maintained in the cortex of prediabetic rats even after daily consumption of white tea (Figure 6, Panel A). However, LDH activity was significantly increased (1.9 ± 0.3 fold variation to Control) in the cortex of prediabetic rats and prediabetic rats consuming white tea (2.3 ± 0.4 fold variation to Control) (Figure 6, Panel B). After lactate is produced, it can be either exported through MCT4 or accumulated in the cells. Our results show that the protein levels of MCT4 in the cortex remained unaltered in prediabetic rats even after daily white tea consumption (Figure 6, Panel C). Interestingly, lactate content was found to be significantly decreased in the cortex of prediabetic rats to 13.8 ± 0.9 nmol/mg of tissue (Figure 6, Panel D), and the daily consumption of white tea was able to decrease even more the cortical lactate content in prediabetic rats to 9.4 ± 0.8 nmol/mg of tissue (Figure 6, Panel D).

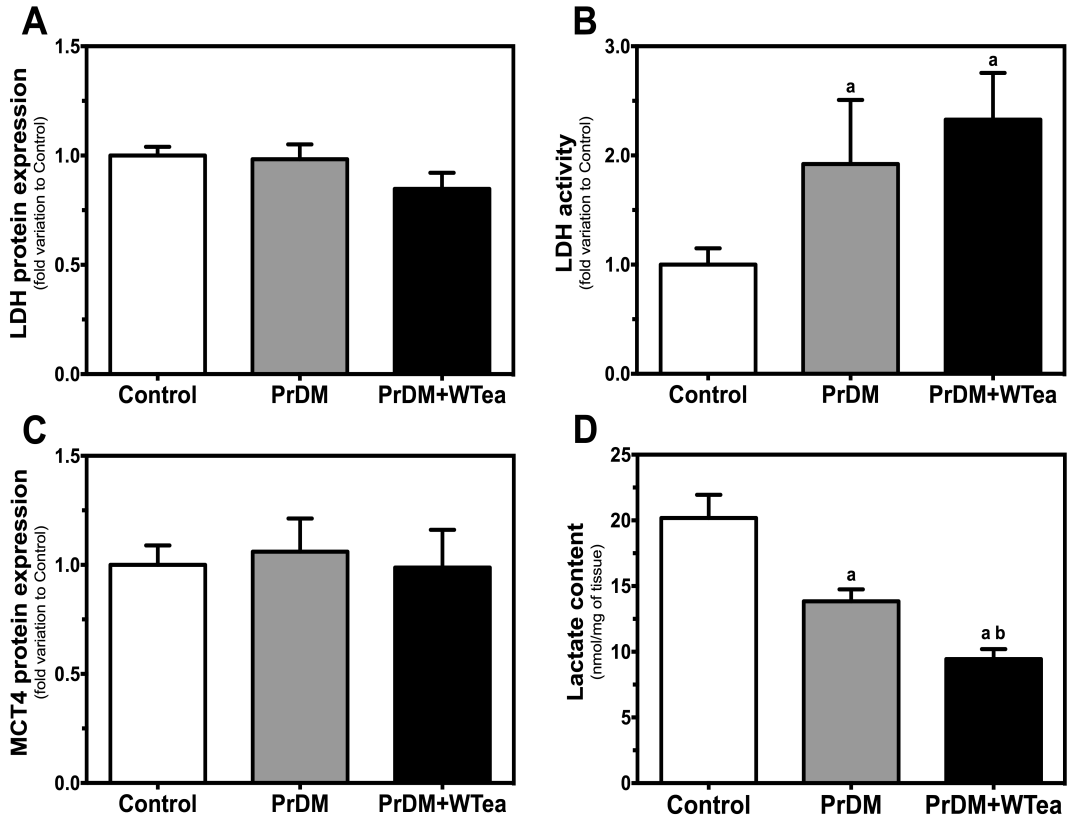


Figure 6 - Effect of daily white tea consumption in lactate dehydrogenase (LDH) and monocarboxylate transporter 4 (MCT4) protein expression, LDH activity and lactate content in cerebral cortex of prediabetic rats. LDH protein expression (Panel A) and activity (Panel B), MCT4 protein levels (Panel C) and lactate content (Panel D) in the cortex of control, prediabetic (PrDM) rats drinking water and PrDM drinking white tea (PrDM+WTea). Results are expressed as means ± SEM (n=6 for each condition). Significantly different results ($P < 0.05$) are indicated as: a - relative to control; b - relative to PrDM.

3. Daily white tea consumption decreases alanine content but was not able to restore lactate/alanine ratio in cerebral cortex of prediabetic rats

Hyperglycemia stimulates glycolysis in the brain. The end-product of glycolysis, pyruvate, can either be metabolized via Krebs cycle, converted to lactate or to alanine. Our results show that alanine content in cerebral cortex is significantly reduced after daily consumption of white tea by prediabetic rats (Figure 7, Panel A). We observed a significant decrease, relatively to control (1.63 ± 0.18 nmol/mg of tissue) and prediabetic (1.67 ± 0.09

nmol/mg of tissue) groups, in the cortex of prediabetic rats consuming white tea (1.14 ± 0.08 nmol/mg of tissue) (Figure 7, Panel A). Interestingly the lactate/alanine ratio, which reflects the reduced nicotinamide adenine dinucleotide (NADH)/oxidized nicotinamide adenine dinucleotide (NAD^+) equilibrium and thus the cellular redox state, was significantly decreased to 7.8 ± 0.4 in the cerebral cortex of prediabetic rats, when comparing with control rats (12.5 ± 0.5) (Figure 7, Panel B). The daily consumption of white tea by prediabetic rats was not able to restore the lactate/alanine ratio in the cortex (Figure 7, Panel B).

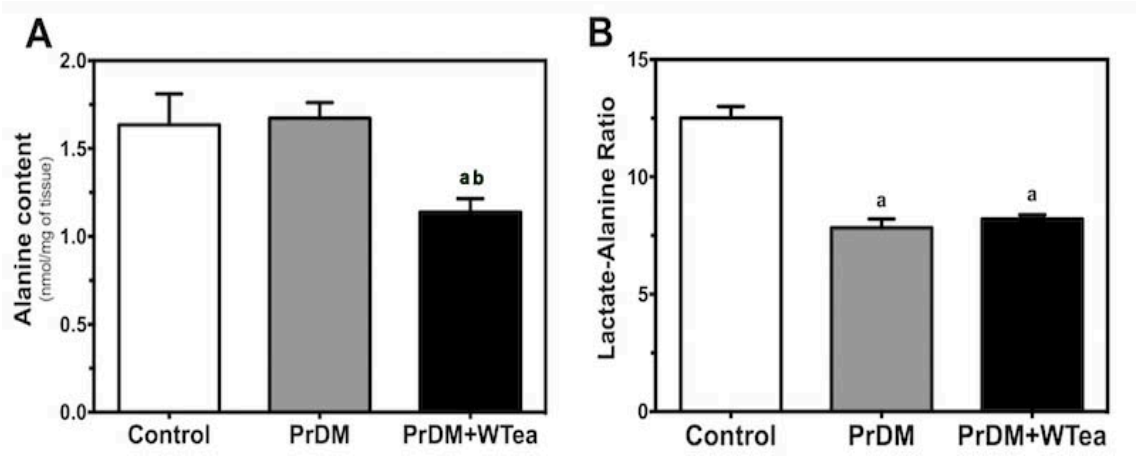


Figure 7 - Effect of daily white tea consumption in alanine content and lactate-alanine ratio in the cerebral cortex of prediabetic rats. Alanine content (Panel A) and lactate-alanine ratio (Panel B) in the cortex of control, prediabetic (PrDM) rats drinking water and prediabetic rats drinking white tea (PrDM+WTea). Results are expressed as means ± SEM (n=6 for each condition). Significantly different results ($P < 0.05$) are indicated as: a - relative to control; b - relative to PrDM.

4. Daily white tea consumption increases the antioxidant capacity and catalase expression, preventing lipid peroxidation and protein oxidation in the cerebral cortex of prediabetic rats

Hyperglycemia is often associated with high OS derived from metabolic alterations. The cortex antioxidant capacity of control, prediabetic and prediabetic rats drinking white tea was measured. As expected, our results show that prediabetes decreases the cortex antioxidant capacity from 20 ± 2 µmol antioxidant potential/mg tissue in control conditions to 16 ± 1 µmol antioxidant potential/mg tissue (Figure 8). Noteworthy, daily consumption of white tea significantly increased cortex antioxidant potential of prediabetic rats to 25 ± 2 µmol antioxidant potential/mg tissue (Figure 8).

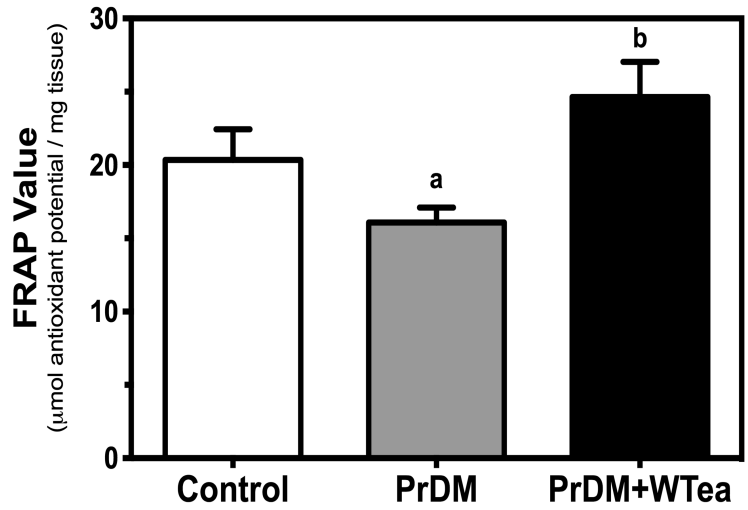


Figure 8 - Effect of daily white tea consumption by prediabetic rats in antioxidant power of cerebral cortex. Ferric reducing antioxidant power (FRAP) in the cortex of control, prediabetic (PrDM) rats drinking water and PrDM drinking white tea (PrDM+WTea). Results are expressed as means ± SEM (n=6 for each condition). Significantly different results ($P < 0.05$) are indicated as: a - relative to control; b - relative to PrDM.

The lipid peroxidation levels in cerebral cortex of control, prediabetic and prediabetic rats drinking white tea were also measured. Our results show that prediabetes significantly increased cortex lipid peroxidation levels from 0.29 ± 0.03 nmol/mg of tissue in control condition to 0.46 ± 0.03 nmol/mg of tissue (Figure 9). Importantly, daily white tea consumption prevented the increase in cortical lipid peroxidation levels of prediabetic rats to 0.26 ± 0.03 nmol/mg of tissue (Figure 9).

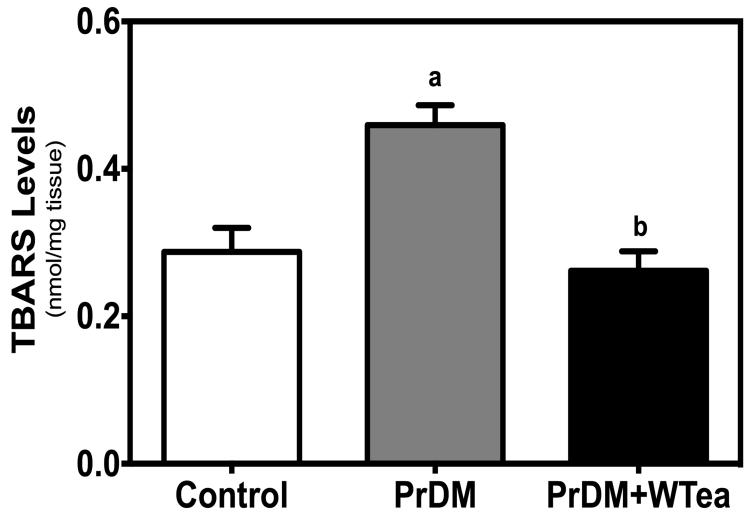


Figure 9 - Effect of daily white tea consumption by prediabetic rats in lipid peroxidation of cerebral cortex. Thiobarbituric acid reactive substances (TBARS) in the cortex of control, prediabetic (PrDM) rats drinking water and PrDM drinking white tea (PrDM+WTea). Results are expressed as means ± SEM (n=6 for each condition). Significantly different results ($P < 0.05$) are indicated as: a - relative to control; b - relative to PrDM.

ROS can induce protein oxidation, which leads to the formation of carbonyl groups. We found that prediabetes significantly increased protein oxidation levels to 1.09 ± 0.07 fold variation to control (Figure 10). Daily consumption of white tea significantly decreased the carbonyl groups content in the cortex when compared to prediabetic rats, but also to the control group (0.89 ± 0.04 fold variation to control) (Figure 10).

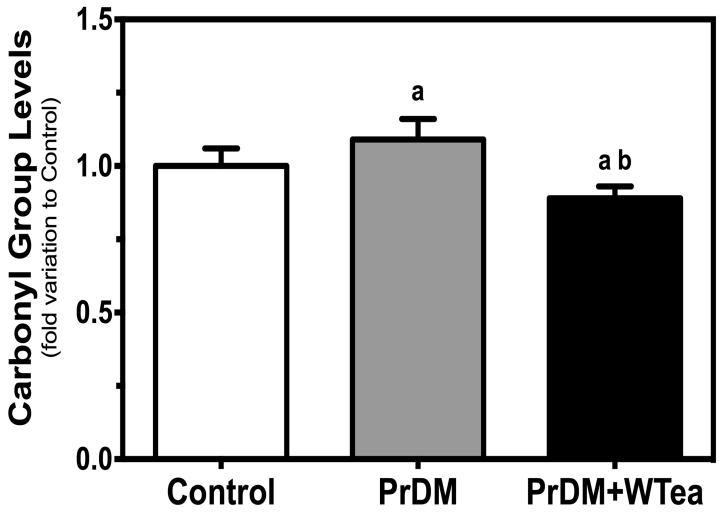


Figure 10 - Effect of daily white tea consumption by prediabetic rats in protein oxidation of cerebral cortex. Carbonyl groups levels in the cortex of control, prediabetic (PrDM) rats drinking water and PrDM drinking white tea (PrDM+WTea). Results are expressed as means ± SEM (n=6 for each condition). Significantly different results ($P < 0.05$) are indicated as: a - relative to control; b - relative to PrDM.

Catalase is one of the major antioxidant defenses present in brain. Our results show that the cortex of prediabetic rats presented a significant decrease of catalase expression to 0.69 ± 0.08 fold variation to control (Figure 11, Panel A). Noteworthy, daily consumption of white tea restored catalase expression level in the cortex of prediabetic rats to 0.96 ± 0.10 fold variation to the control (Figure 11, Panel A). Other important cellular antioxidant mechanisms are exerted by glutathione (for review see (Niedowicz and Daleke 2005)). Our results show that total glutathione content was reduced in the cortex of prediabetic rats to 0.11 ± 0.01 nmol/mg of tissue and daily white tea consumption was not able to restore total glutathione content in the cortex of prediabetic rats (Figure 11, Panel B). No alterations were detected in GSSH content in the cortex of prediabetic and prediabetic rats drinking white tea, when comparing with control rats (Figure 11, Panel B). However, GSH content was significantly decreased to 0.03 ± 0.01 nmol/mg tissue in the cortex of prediabetic rats and 0.04 ± 0.01 nmol/mg tissue in the cortex of prediabetic rats that consuming white tea, relatively to control (0.08 ± 0.02 nmol/mg tissue) (Figure 11, Panel B).

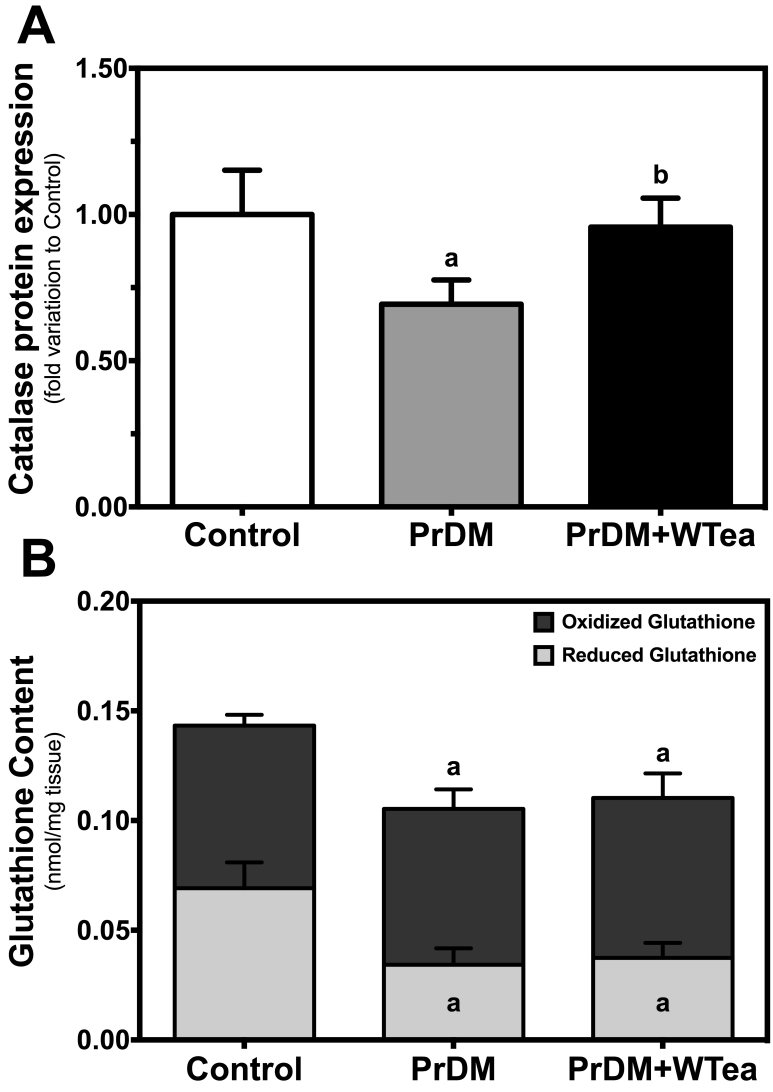


Figure 11 - Effect of daily white tea consumption in catalase protein expression and glutathione content in the cerebral cortex of prediabetic rats. Catalase protein expression (Panel A) and glutathione content (panel B) in the cortex of control, prediabetic (PrDM) rats drinking water and prediabetic rats drinking white tea (PrDM+WTea). Results are expressed as means ± SEM (n=6 for each condition). Significantly different results ($P < 0.05$) are indicated as: a - relative to control; b - relative to PrDM.

5. Daily white tea consumption restores valine content in the cerebral cortex of prediabetic rats

Our results show that there are no differences in the levels of glutamate, aspartate and taurine in the brain of control and prediabetic rats drinking water or white tea (Table 2). Concerning to NAA levels in the cerebral cortex of control, prediabetic and prediabetic rats after white tea consumption, our results show that prediabetes significantly decreased the levels of NAA in the cortex to 3.4 ± 0.1 nmol/mg of tissue compared to 4.6 ± 0.5 nmol/mg of tissue in control rats. Choline was increased in the cortex of prediabetic rats and prediabetic rats drinking white tea (3.5 ± 0.1 and 3.5 ± 0.3 nmol/mg of tissue, respectively), when compared with control rats (1.6 ± 0.2 nmol/mg of tissue) (Table 2). Thus, white tea appears to mediate its beneficial effects by neurotransmission-independent mechanisms. Concomitantly, our results show that in the cortex of prediabetic rats and prediabetic rats drinking white tea, the levels of GABA, which is a primary inhibitor of neurotransmission, remain unaltered when compared with the control (Table 2). Interestingly, daily white tea consumption restored valine levels, a crucial aminoacid involved in protein synthesis, in the cerebral cortex of prediabetic rats to control values. The valine levels in the cortex of control rats was 0.26 ± 0.02 nmol/mg of tissue, that significantly increased to 0.36 ± 0.03 nmol/mg of tissue in the cortex of prediabetic rats and was restored after daily white tea consumption (0.26 ± 0.02 nmol/mg of tissue; Table 2).

Table 2 - Levels of some metabolites in the cortex of control, prediabetic (PrDM) rats drinking water and prediabetic rats drinking white tea (PrDM+WTea). Results are expressed as means ± SEM (n=6 for each condition). Significantly different results ($P < 0.05$) are indicated as: a - relative to control; b - relative to PrDM.

Metabolite (nmol/mg of tissue)	Control	PrDM	PrDM+WTea
Glutamate	6.6 ± 0.7	6.6 ± 0.1	7.3 ± 0.9
NAA	4.6 ± 0.5	3.4 ± 0.1 [a]	3.6 ± 0.6
Aspartate	2.6 ± 0.2	2.7 ± 0.3	2.5 ± 0.2
Choline	1.6 ± 0.2	3.5 ± 0.1 [a]	3.5 ± 0.3 [a]
GABA	5.0 ± 0.7	5.0 ± 0.3	5.0 ± 0.5
Taurine	3.6 ± 0.6	4.3 ± 0.1	4.8 ± 0.4
Valine	0.26 ± 0.02	0.36 ± 0.03 [a]	0.26 ± 0.02 [b]

V. Discussion

Tea (*C. sinensis*) is a medicinal plant that deserves special merit since it is the second most consumed beverage in the world and has been reported to possess several health benefits (for review see (Dias, T. R. et al., 2013)). A complex mixture of bioactive phytochemicals composes tea and non-fermented teas, such as white tea, and thus, they are considered good sources of natural antioxidants. Recently, we have demonstrated that white tea has higher concentration of polyphenols than green tea, which results in greater antioxidant activity (Dias, T. R. et al., 2014). TP seem to be the most important component of white tea leaves, due do their relative abundance, constituting about 30% of their dry weight, with good bioactive properties (for review see (Dias, T. R. et al., 2013)). Due to its formation process, white tea contains relatively high concentrations of catechins. In fact, the concentration of polyphenols, caffeine, gallic acid and others, is significantly higher in white than green tea (Hilal, Y. and Engelhardt, U. 2007). Thus, the possible increased antioxidant activity of white tea could be related to higher concentrations of several of the major constituents explaining its benefits to health. Several studies have reported that tea phenolic compounds, mainly catechins, are potent antioxidant agents, scavenging ROS (Nakagawa, T. and Yokozawa, T. 2002) and metal chelators (Atoui, A. K. et al., 2005). Continuous administration of EGCG for 30 days was able to significantly improve rat brain antioxidant defenses, ameliorating the age-induced OS (Srividhya, R. et al., 2008).

Besides polyphenols, white tea contains other compounds with considerable interest for human health such as caffeine and L-theanine (Martins, A. D. et al., 2014). Caffeine is a likely candidate against memory loss (for review see (Cunha, R. A. 2008)) and has a great neuroprotective potential (Duarte, J. M. et al., 2009). Interestingly, white tea contains higher amount of caffeine than green tea (Dias, T. R. et al., 2014), and the consumption of caffeine-containing beverages, in particular tea, is associated with a lower risk of developing T2DM (Dieren, S. v. et al., 2009). In turn, L-theanine is an antioxidant that is reported to prevent neuronal death and loss of memory (Kim, T. I. et al., 2009). However, the number of studies regarding white tea consumption is so far negligible and the underlying mechanisms of action remain largely unknown.

The brain is very susceptible to glucose fluctuations and hyperglycemia-induced OS (Bree, A. J. et al., 2009, Cardoso, S. et al., 2010), particularly the cerebral cortex, which may cause several deleterious effects on brain function. Thus, herein we studied the effect of the daily consumption of white tea in the cerebral cortex of STZ-induced prediabetic rats. STZ-treated newborn rats developed typical prediabetes features. Blood glucose levels were mildly elevated, not enough to meet the criteria for T2DM establishment, and the rats developed glucose intolerance and insulin insensitivity. Although daily consumption of white tea was not able to decrease blood glycemia to normal levels, it significantly improved glucose tolerance and insulin sensitivity. TP have been suggested as possible responsible for these actions. In fact, the capacity of TP to improve glucose and insulin

sensitivity in hyperglycemia conditions has been reported in *in vitro* studies (Snoussi, C. et al., 2014), animal experiments (Sabu, M. C. et al., 2002) and even in clinical observations (Hosoda, K. et al., 2003). There are several mechanisms that may mediate these effects, but they are suggested as being primarily mediated by the strong antioxidant action of TP (Sabu, M. C. et al., 2002). It is described that TP can be responsible by improvement of glucose homeostasis probably through modulations of intestinal absorption of nutrients (Snoussi, C. et al., 2014), and inhibition of intestinal glucose uptake by sodium dependent glucose transporter (Kobayashi, Y. et al., 2000). Green tea extracts and green tea catechins such as EGCG alone have also been reported to decrease blood glucose (Roghani, M. and Baluchnejadmojarad, T. 2010), although the mechanisms remain largely unknown.

Mild hyperglycemia is a characteristic feature of prediabetes and is associated with complications in several organs, including the brain (Roriz-Filho, J. S. et al., 2009, Santos, R. X. et al., 2014a). Glucose levels in the brain are very distinct from blood glucose levels and thus, its metabolism is crucial for proper brain functioning (for review see (Routh, V. H. 2002)). Within the brain, the cortex has been reported as very sensitive to hyperglycemia (Bree, A. J. et al., 2009, Cardoso, S. et al., 2010). Extracellular glucose enters in the cerebral cortex through the action of GLUTs. Daily consumption of white tea decreased GLUTs levels in cortex of prediabetic rats. The inhibition of glucose transport through modulation of GLUTs expression has been attributed to TP. It has been reported that intestinal glucose uptake is inhibited by TP through competitive inhibition of GLUTs (Kobayashi, Y. et al., 2000, Shimizu, M. et al., 2000). Moreover, *in vitro* studies in Sertoli cells, which are metabolically active cells (for review see (Oliveira, P. F. et al., 2014)), also showed that white tea extract modulates GLUTs expression (Martins, A. D. et al., 2014). Prediabetic rats drinking white tea presented reduced GLUT1 expression when compared to prediabetic rats drinking water as well as decreased GLUT3 compared to prediabetic rats drinking water and control rats. Thus, reduced brain GLUTs expression in prediabetic rats drinking white tea may explain the persistent hyperglycemia though glucose tolerance is significantly improved. The decrease of GLUTs may lead to a lower amount of glucose entering in brain tissue increasing its concentration in the periphery, which may explain the persistent hyperglycemia after daily white tea consumption.

After glucose reaches the cortex, it is metabolized via glycolysis. Although no changes were detected in the cortical levels of PFK-1 (that catalyzes the first rate-limiting step in the glycolytic metabolism) of prediabetic rats and prediabetic rats consuming white tea. Following the decrease in GLUTs levels it could be expected a decrease in PFK-1 protein levels illustrating an inhibition of glucose metabolism in cerebral cortex induced by the consumption of white tea. Besides, green tea extract is also reported to inhibit intestinal lipases (Juhel, C. et al., 2000), and tea catechins are also known to inhibit enzymes, such as lactase, involved in carbohydrate digestion (Naz, S. et al., 2011). Of note,

we studied the expression of PFK-1 but its activity may be altered thus, the measurement of PFK-1 activity in the future may elicit a mechanism by which the consumption of white tea changes the glycolytic profile of cerebral cortex. On the other hand, prediabetic rats drinking water and white tea presented a significant increase in LDH activity, which illustrates the stimulation of glycolysis. Interestingly, lactate content was found to be decreased in the cortex of prediabetic rats and further decreased after daily consumption of white tea. Lactate is an important energy substrate for neurons during activation (Alessandri, B. et al., 2012). Moreover, lactate may be a crucial metabolic fuel for the cerebral cortex under stressful conditions (for review see (Schurr, A. 2006)). Thus, as expected, moderate hyperglycemia in prediabetic rats leads to less lactate accumulation in the cortex due to an increase in its metabolism. This was in accordance with the increase of LDH activity that operates in both ways (promoting lactate production or lactate degradation). Moreover, this is also concomitant with the maintenance of MCT4 levels, which indicates that although glycolysis is stimulated, the lactate produced is metabolized instead of being exported. Interestingly, daily white tea consumption decreased alanine content in the cerebral cortex of prediabetic rats, which illustrate a metabolic adaptation to the decrease of lactate content. The lactate/alanine ratio reflects the intracellular redox state since the conversion of pyruvate to lactate or its conversion to alanine is coupled with re-oxidation of NADH into NAD+ (O'Donnell, J. M. et al., 2004). Our results suggest that white tea intake by prediabetic rats is able to partly restore the intracellular redox state, as can be seen by the increased lactate/alanine ratio, even though not reaching control condition values.

Glycemia fluctuations, particularly hyperglycemia, can amplify brain OS inducing several alterations. Within the brain, the cerebral cortex is particularly vulnerable to ROS production, since it has a limited antioxidant capacity (for review see (Wang, X. and Michaelis, E. K. 2010)). As expected, prediabetes reduced the antioxidant capacity and increased cortical oxidative damage, as evaluated by lipid peroxidation and protein oxidation levels. Moreover, our results show that catalase expression, which is one of the most important enzymatic defenses in the cortex, is reduced in prediabetes rats. Besides that, total glutathione and GSH content, usually used as a measure of a balance between ROS production and antioxidant defenses, was also found to be decreased in the cerebral cortex of prediabetic rats. There is a high interest in natural products that can control hyperglycemia and OS. Antioxidant potential of white tea, potentiated by its polyphenols content, make it a very effective ROS scavengers. Our results show that daily consumption of white tea was able to restore to control values the cerebral cortex antioxidant capacity and lipid peroxidation levels of prediabetic rats. Noteworthy, in those rats, the protein oxidation levels were significantly decreased even when compared to control values. Of note, recent studies reported a neuroprotective effect of tea extracts with low content in

EGCG (Rodrigues, J. et al., 2013), particularly against lipids and proteins oxidation, illustrating that other components can also play a crucial neuroprotective role.

In our experiments, the metabolic and oxidative status of cerebral cortex was altered in prediabetic rats and thus, as expected, several intracellular metabolites were also found to be altered. NAA is very abundant in CNS and can act as a marker of cerebral damage (Ariyannur, P. S. et al., 2010); it can also be a stored form of aspartate. It has been reported that NAA levels are decreased in brain of diabetic patients (Sinha, S. et al., 2014). Our results also show that NAA levels are decreased in the cerebral cortex of prediabetic rats and that the daily consumption of white tea by prediabetic rats increases the levels of NAA in the cortex. Since decreased NAA levels as a result of chronic hyperglycemia are usually associated with decreased neuronal viability, our results show that daily white tea consumption is able to improve this condition. This effect appears to be independent of aspartate metabolism since no alterations were detected in the levels of aspartate in the cerebral cortex of prediabetic rats drinking water or white tea. Interestingly, the levels of GABA remained unaltered but the levels of choline, which is essential for neurotransmitters synthesis, were found to be increased in the cortex of prediabetic rats. This is in accordance with other works that reported an increase in the levels of choline in the brain of diabetic patients (Geissler, A. et al., 2003). Even though, white tea consumption was not able to restore the cortical choline levels in prediabetic rats. On the other hand, the daily consumption of white tea restored valine levels in the cerebral cortex of prediabetic rats to normal levels. Valine is a crucial metabolite involved in protein synthesis and our results demonstrate that daily white tea consumption decreases protein oxidation and restores protein synthesis in the brain cortex of prediabetic rats.

Undoubtedly, impaired glucose metabolism induces OS that contributes to brain damage. Although daily white tea consumption did not decreased blood glycemia levels, it improved glucose tolerance and insulin sensitivity in prediabetic rats. Moreover, daily white tea consumption altered the brain cortical glycolytic profile of prediabetic rats. The effect was particularly evident in decreasing GLUTs expression and the content of lactate and alanine. These changes were followed by a clear improvement in cerebral cortex oxidative status. Overall, cortex of prediabetic rats drinking white tea presented a greater antioxidant capacity and normalized expression of catalase.

As discussed, the protective effect of white tea consumption is mediated not only by the sum of the action of isolated compounds but by the synergistic action of a mixture of compounds. For instance, studies that associate coffee consumption with reduced risk of some types of cancer show that those effects may be due to the synergistic effect of all the compounds comprised in coffee, and not to a single compound in particular, such as

caffeine (for review see (Nkondjock, A. 2009)). In addition, many studies shown that the long-term consumption of several tea phytochemicals together can result in better antimicrobial benefits than the action of single components (for review see (Friedman, M. 2007)). However, more studies are needed to unveil the mechanisms responsible for the synergistic action of the compounds present in tea. Moreover, in usual dietary practice, tea is generally taken at approximately 1% (w/v), but bioavailability of TP is poor (for review see (Khan, N. and Mukhtar, H. 2007)). On the other hand, the amounts of tea consumed by humans are much lower than the amounts given to animals. The differences between the animal species subjected to research and humans may also hamper the correct interpretation, extrapolation and practical application of the results and conclusion. Thus, more studies are needed.

Overall, our results suggest that daily consumption of white tea by prediabetic subjects may improve cerebral cortex metabolic and oxidative profile. Currently, tea and its phytochemicals (particularly catechins) are under debate since several health benefits have been reported. However, more studies are needed to unravel the mechanisms associated with the antioxidant, antidiabetic and neuroprotective tea effects on the organism, particularly in the brain.

VI. Conclusions

DM is a pandemic disease affecting an enormous number of people worldwide. Prediabetes, a prodomal stage of DM, is increasing among young people and is associated with a high risk of development of T2DM. Several organs are affected by hyperglycemia, such as the brain, particularly the cerebral cortex.

Undoubtedly, impaired glucose metabolism induces OS that contributes to brain damage. Our results show that daily white tea consumption: (1) did not decreased blood glycemia levels, but improved glucose tolerance and insulin sensitivity in prediabetic rats; (2) altered the glycolytic profile of cerebral cortex of prediabetic rats. This effect was particularly evident in GLUTs expression and lactate and alanine content; (3) improved the cortex oxidative status of prediabetic rats; (4) significantly increased the antioxidant power and normalized the expression of catalase in the cortex of prediabetic rats. As discussed, the protective effects of white tea consumption are mediated not only by the sum of action of isolated compounds but probably by the synergistic action of the mixture of compounds.

Nowadays the interest for traditional medicine, which relies on the use of natural products, including teas, is increasing. It is urgent to find new and effective preventive or therapeutic strategies to ameliorate the complications induced by hyperglycemia. Tea consumption, namely white tea, appears to be a good candidate.

Herein, we report for the first time the protective effects of the consumption of white tea in the prediabetic cerebral cortex supporting that white tea can represent an alternative co-adjuvant in the treatment of DM and/or prevent DM-related dysfuction brain cortex. However, more studies are needed to unravel the role of white tea and its phytochemicals in the protection against the injury caused by prediabetes in the brain.

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VIII. Annex I

List of publications resultant from the work developed during the M.Sc. in Biomedical Sciences:

Nunes AR, Alves MG, Moreira PI, Oliveira PF, Silva BM (2014) Can tea consumption be a safe and effective therapy against diabetes mellitus-induced neurodegeneration? (Submitted)

Nunes AR, Alves MG, Tomás GD, Conde VR, Cristóvão AC, Moreira PI, Oliveira PF, Silva BM (2014) Daily consumption of white tea (*Camellia sinensis* (L.)) by prediabetic Wistar rats improves cerebral cortex metabolic and oxidative profile. (Submitted)