



UNIVERSIDADE DA BEIRA INTERIOR  
Ciências da Saúde

# Efeito do Consumo do Chá Branco no Coração de Ratos Diabéticos Tipo 2

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# Effect of White Tea Consumption on the Heart of Type 2 Diabetic Rats

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Master degree Thesis in Biomedical Science

**Ciências Biomédicas**

(2<sup>nd</sup> cycle of studies)

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

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*(Nelson Augusto Ferreira Teixeira)*

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## Resumo

De acordo com a Organização Mundial de Saúde (WHO), a diabetes mellitus (DM) é a nona causa de morte a nível mundial e cerca de 80% destas mortes são devido a doenças cardiovasculares. O coração funciona como uma bomba e tem uma necessidade contínua de energia, sendo muito susceptível a alterações metabólicas e/ou oxidativas. É bem conhecido que a DM é responsável por alterações metabólicas importantes que resultam num aumento de stress oxidativo (OS). Muitas plantas medicinais são usadas por todo o mundo para contrariar os efeitos deletérios da DM. De facto, alguns efeitos prejudiciais da DM são prevenidos pelo uso dessas plantas, mas os mecanismos pelos quais essa prevenção ocorre, permanecem desconhecidos. Nos últimos anos tem-se assistido a um aumento significativo de estudos científicos baseados no consumo de chá, uma das bebidas mais consumidas no mundo. Existem vários estudos que demonstram que o consumo de chá pode melhorar a função cardíaca de indivíduos diabéticos. No entanto, a maioria destes estudos está focada no chá verde (GT) e os mecanismos de acção do chá permanecem desconhecidos. Neste trabalho colocámos a hipótese de que o consumo de chá branco (WTEA), que é o chá menos estudado, poderia melhorar o funcionamento do coração de ratos diabéticos. Para testar a nossa hipótese usámos um modelo de rato para a diabetes tipo 2 (T2D). Os animais foram divididos em 3 grupos: grupo controlo, grupo T2D induzido por estreptozotocina (STZ) e grupo T2D induzido por STZ ao qual foi administrado chá branco (STZ+WTEA). Antes do sacrifício, os animais foram sujeitos aos testes de tolerância à glucose e resistência à insulina. Após recolha do tecido cardíaco, os níveis de peroxidação lipídica e de oxidação proteica foram determinados. O conteúdo de glucose, lactato, alanina e acetato nos corações foi quantificado. Os níveis de mRNA do transportador de glucose 1 (GLUT1), lactato desidrogenase (LDH) e transportador de monocarboxilatos 4 (MCT4) foram igualmente determinados, assim como a actividade da LDH.

Os resultados obtidos demonstram que o consumo regular de WTEA permitiu a recuperação da sensibilidade à insulina e da tolerância à glucose nos ratos T2D. Além disso, preveniu a peroxidação lipídica e diminuiu a oxidação proteica nos corações de ratos T2D. Esses ratos apresentaram distúrbios severos no processo glicolítico que foram normalizados pelo consumo de WTEA. Para isso contribuiu um efeito importante na regulação da expressão do GLUT1 e na actividade da LDH.

Mais estudos são necessários para confirmar os efeitos benéficos do consumo regular de WTEA mas os nossos resultados demonstram que a ingestão de chá branco pode ser uma boa estratégia para diminuir os efeitos nefastos da T2D na função cardíaca.

## **Palavras-Chave:**

*Diabetes Mellitus*, Chá Branco, *Camellia sinensis*, Doenças cardiovasculares, Stress oxidativo.

## Resumo Alargado

De acordo com a Organização Mundial de Saúde (WHO), a diabetes mellitus (DM) é a nona causa de morte no mundo e cerca de 80% destas mortes são devidas a doenças cardiovasculares. Actualmente, o número de indivíduos com diabetes tipo 2 (T2D) tem vindo a aumentar para números pandémicos, especialmente devido aos hábitos alimentares e ao estilo de vida. Vários investigadores têm apontado como principal causa de morte nas sociedades modernas e em desenvolvimento, as doenças metabólicas relacionadas com distúrbios na manutenção dos níveis plasmáticos de glicose. O coração funciona como uma bomba e tem uma necessidade energética contínua, necessitando de uma quantidade basal de energia elevada para manter a actividade contráctil. Por esse motivo, é um órgão altamente susceptível a alterações metabólicas e/ou oxidativas. É bem conhecido que a DM é responsável por alterações metabólicas importantes, nomeadamente pelo aumento dos níveis de glicose na corrente sanguínea que, tal como acontece em outros órgãos, chega ao coração através de difusão resultando num aumento da actividade metabólica e, conseqüentemente, num aumento do stress oxidativo (OS). Em todo o mundo, muitas são as plantas medicinais usadas para contrariar os efeitos deletérios da DM. De facto, algumas características prejudiciais da DM são prevenidas pelo uso dessas plantas, embora os mecanismos pelos quais são conseguidos esses melhoramentos permaneçam desconhecidos. Nos últimos anos tem-se assistido a um aumento significativo de estudos científicos baseados no consumo de chá. O chá é usado como um produto medicinal há centenas de anos e é, ainda nos dias de hoje, uma das bebidas mais populares e consumidas no mundo. Existem vários estudos que demonstram que o consumo de chá, devido à sua actividade antioxidante, pode melhorar a tolerância à glucose e a sensibilidade à insulina em indivíduos diabéticos. Tem ainda sido sugerido que o consumo de chá pode melhorar a função cardíaca desses indivíduos. No entanto, muitos destes estudos são focados no chá verde (GT) e os mecanismos envolvidos na prevenção dos efeitos nefastos da DM permanecem desconhecidos. Neste trabalho, começámos por determinar o poder antioxidante de extractos de GT e chá branco (WTEA), através do teste do potencial antioxidante férrico-redutor (FRAP), e concluímos que o WTEA possuía um potencial antioxidante muito superior. Assim, colocámos a hipótese de que o consumo de chá branco, sendo o chá menos processado e com maior potencial antioxidante, poderia melhorar alguns parâmetros metabólicos corporais e do tecido cardíaco em ratos diabéticos. Para testar a nossa hipótese usámos um modelo de rato para a diabetes tipo 2 (T2D). Os animais foram divididos em 3 grupos: grupo controlo, grupo T2D induzido por estreptozotocina (STZ) e grupo T2D induzido por STZ ao qual foi administrado WTEA (STZ+WTEA). Antes do sacrifício, os animais foram sujeitos aos testes de tolerância à glucose e resistência à insulina. O tecido cardíaco foi então sujeito ao teste FRAP para determinar o potencial antioxidante nos corações dos animais dos vários grupos. A peroxidação lipídica e a oxidação de proteínas foram determinadas quantificando as espécies reactivas ao ácido tiobarbitúrico (TBARS) e os

níveis de grupos carbonilo, respectivamente. O conteúdo de glicose, lactato, alanina e acetato nos corações foi determinado por ressonância magnética nuclear (NMR). Os níveis de mRNA do transportador de glicose 1 (GLUT1), lactato desidrogenase (LDH) e transportador de monocarboxilatos 4 (MCT4) no tecido cardíaco foram determinados por RT-PCR. A actividade da LDH foi medida espectrofotometricamente.

Os nossos resultados demonstram que o consumo regular de WTEA permitiu a recuperação da sensibilidade à insulina e da tolerância à glucose nos ratos com T2D. Os corações dos ratos com T2D apresentavam menor poder antioxidante que os do grupo controlo e o consumo de WTEA permitiu recuperar o poder antioxidante. Além disso, o consumo de WTEA preveniu a peroxidação lipídica e diminuiu a oxidação proteica nos corações de ratos com T2D. Esses ratos apresentaram ainda distúrbios severos no processo glicolítico que foram prevenidos pelo consumo de WTEA, nomeadamente no que diz respeito ao conteúdo de lactato e de acetato, que foram restaurados para valores mais próximos dos normais. Para isso contribuiu um efeito importante na expressão do GLUT1 e na actividade da LDH, ambas normalizadas pelo consumo de WTEA, no tecido cardíaco de ratos com T2D.

Mais estudos serão então necessários para confirmar os efeitos benéficos do consumo regular de WTEA, mas os nossos resultados demonstram que beber WTEA pode ser uma estratégia eficaz, segura e económica para diminuir os efeitos nefastos da T2D na função cardíaca.

## **Abstract**

According to the World Health Organization (WHO), diabetes mellitus (DM) is the ninth leading cause of death worldwide, and 80% of those deaths are due to cardiovascular disease. The heart functions as a pump with a continuous need for energy and therefore is very susceptible to metabolic and/or oxidative alterations. It is well known that DM induces important metabolic alterations that result in increased oxidative stress (OS). Many medicinal plants are used worldwide to counteract the deleterious effects of DM. Although it is a fact that some harmful characteristics of DM are indeed ameliorated, the mechanisms by which medicinal plants improve the body response to this disease remain unknown. In the last few years, scientific studies concerning the effect of tea consumption, one of the most consumed beverages in the world, significantly increased. There are several works reporting that tea consumption may improve heart function in diabetic individuals. However, much of these studies are focused in green tea (GT) and most of the mechanisms of tea action remain unknown. Herein, we hypothesized that the consumption of the less studied tea, the white tea (WTEA), improves the heart functioning of diabetic individuals. To test our hypothesis, we used a STZ-induced Type 2 diabetes (T2D) rat model. The animals were divided in 3 groups: control, STZ-induced T2D (STZ) and WTEA drinking STZ-induced T2D rats (STZ+WTEA). Before sacrifice, the animals were subjected to a glucose tolerance and insulin sensitivity tests. Heart lipid peroxidation and protein oxidation were determined. Glucose, lactate, alanine and acetate contents in the hearts were quantified. mRNA expression levels of glucose transporter-1 (GLUT1), lactate dehydrogenase (LDH) and monocarboxylate transporter 4 (MCT4) were also determined, as well as LDH activity, in the heart.

Our results show that WTEA consumption restored insulin sensitivity and glucose tolerance in STZ-induced T2D rats. Besides, WTEA consumption restored lipid peroxidation to control values and decreased protein oxidation in hearts from STZ-induced T2D rats. Finally, STZ-induced T2D rats presented an impaired glycolysis that appears to be ameliorated by WTEA consumption. In these processes, regulation of GLUT1 and LDH activity proved to be essential in the protective effect shown by WTEA consumption.

More studies are needed to confirm the beneficial effects of WTEA consumption but our results provide clear evidence that WTEA ingestion can be a good, safe and inexpensive strategy to decrease the deleterious effects of T2D to the heart.

## Keywords:

Diabetes Mellitus, White tea, *Camellia sinensis*, Cardiovascular diseases, Oxidative stress.

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## **List of Abbreviations**

**AGE** - Advanced glycation end-products  
**ADP** - Adenosine diphosphate  
**ATP** - Adenosine triphosphate  
**AUC** - Area under the curve  
**BMI** - Body mass index  
**Cs** - Catechins  
**CAC** - Citric acid cycle  
**CO<sub>2</sub>** - Carbon dioxide  
**COMT** - Catechol-O-methyltransferase  
**CVD** - Cardiovascular diseases  
**DM** - Mellitus diabetes  
**DPPH** - Diphenylpicrylhydrazyl  
**DNPH** - 2,4-Dinitrophenylhydrazine  
**DNA** - Deoxyribonucleic acid  
**EC** - Epicatechin  
**ECG** - Epicatechin gallate  
**EGC** - Epigallocatechin  
**EGCG** - Epigallocatechin 3-gallate  
**FADH<sub>2</sub>** - Flavin adenine dinucleotide  
**FRAP** - Ferric reducing antioxidant power  
**GLUT1** - Glucose transporter type 1  
**GT** - Green tea  
**HDL** - high density lipoprotein  
**HDL<sub>c</sub>** - cholesterol High density lipoprotein  
**IDF** - International Diabetes Federation  
**INT** - tetrazolium salt  
**LDL** - Low density lipoprotein  
**LDL<sub>c</sub>** - Cholesterol low density lipoprotein  
**LDH** - Lactate dehydrogenase  
**LDHA** - Lactate dehydrogenase A  
**MCT4** - monocarboxilase transporter 4

**MDA** - Malondialdehyde  
**NAD<sup>+</sup>** - Nicotinamide adenine dinucleotide  
**NADPH** - Nicotinamide adenine dinucleotide phosphate  
**NMR** - nuclear magnetic resonance  
**NO** - Nitric oxide  
**OADs** - Oral Anti-diabetic drugs  
**OS** - Oxidative stress  
**PBS** - Phosphate buffered saline  
**RT-PCR** - Real time polymerase chain reaction  
**PEP** - Phosphoenolpyruvate  
**PFK** - Phosphofruktokinase  
**PK** - Piruvate kinase  
**PPO** - Polyphenol oxidase  
**PST** - Phenolsulfotransferase  
**RNA** - Ribonucleic *acid*  
**ROS** - Reactive oxygen species  
**RONS** - Reactive oxygen and nitrogen species  
**SDS** - Sodium dodecyl sulfat  
**STZ** - Streptozotocin  
**T1D** - Type 1 diabetes mellitus  
**T2D** - Type 2 diabetes mellitus  
**TBA** - Thiobarbituric acid  
**TBARS** - Thiobarbituric acid reactive substances  
**TPTZ** - 2,4,6-tripyridyl-s-Triazine  
**UGT** - UDP-glucuronosyltransferase  
**WHO** - World Health Organization  
**WHR** - Waist - to hip-ratio  
**WTEA** - White tea



# **I . Introduction**

# 1. Diabetes Mellitus

Diabetes mellitus (DM) is a life lasting, devastating, expensive, manageable but incurable disease that is very common nowadays. There was an estimated 285 million adults with DM in 2010 and by 2030 this number will raise to 439 million, due to aging and growth of the population, increase in obesity and sedentary lifestyle (Shaw, Sicree, & Zimmet, 2010). However, the diagnostic of obesity and DM is raising in young individuals (Blanck et al., 2006; Wild, Roglic, Green, Sicree, & King, 2004), thus these numbers may be underestimated. According to (Zhang et al., 2010), the global expenditure of DM in 2010 was of USD 376 billion, imposing a large economic burden on the national health care systems.

This disease consists in a chronic metabolic disorder, characterized by a state of insulin deficiency that leads to hyperglycemia (Gupta et al., 2005) inducing changes in the metabolism of carbohydrates, lipids and proteins (Da Poian & de Carvalho-Alves, 2005; Negri, 2005), defects in the reactive oxygen species (ROS) scavenging enzymes (Kesavulu, Giri, Rao, & Apparao, 2000), and increases the oxidative stress (OS) impairing pancreatic beta cells (Baliga & Sapsford, 2009; Hamden, Jaouadi, Carreau, Aouidet, & Elfeki, 2011). Increased OS has been related with the pathogenesis of DM (Kaushik, Satya, Khandelwal, & Naik, 2010). Moreover, hyperglycemia-induced protein glycation generates high amounts of superoxide free radicals (Atalay & Laaksonen, 2002; Cunningham, Leffell, Mearkle, & Harmatz, 1995; Lipinski, 2001; Memisoğullari, Taysı, Bakan, & Capoglu, 2003; Raskin et al., 2000). The generation of active ROS may lead to lipid peroxidation and the creation of reactive products, which induces damages in cell molecules and structures (Kaushik et al., 2010). These processes are responsible for increasing chances of developing cardiovascular diseases (CVD).

There are mainly two types of DM: type 1 (T1D) and type 2 (T2D). T1D 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. Thus, T1D patients need exogenous insulin administration and are well known for being insulin dependent. T2D is characterized by a progressive impairment of insulin secretion by the  $\beta$ -cells from the pancreas and by a relative decline sensitivity of target tissues to the action of the hormone (Remy Burcelin et al., 1999; Cleeman, Grundy, Becker, & Clark, 2001). These patients are not insulin dependent and face pharmacological treatment. They represent almost 90% of DM cases worldwide. DM is characterized by high blood glucose, polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger), weight loss, blurred vision, nausea and vomiting, weakness and tiredness, irritability, mood changes (Modak, Dixit, Londhe, Ghaskadbi, & Devasagayam, 2007), reduced plasma antioxidant levels (Facchini, Hua, Reaven, & Stoohs, 2000), among other characteristics.

One of the major causes of mortality in diabetic patients are the CVD. Several reports suggest that the majority of people with T2D may die from CVD (Boccarda & Cohen, 2004; Morrish, Wang, Stevens, Fuller, & Keen, 2001). Hypertension is almost twice as frequent in diabetic

individuals comparatively to non-diabetic (Sowers, Epstein, & Frohlich, 2001). Other important risk factor for CVD of these individual, beside insulin resistance, are: obesity, atherosclerosis, dyslipidemia, low plasma levels of HDL, microalbuminuria, endothelial dysfunction, platelet hyperaggregability and coagulation abnormalities (Sowers et al., 2001; Wolfram, 2007). These parameters often appear clustered in diabetic patients so that several international organizations such as the World Health Organization (WHO) and the, International Diabetes Federation (IDF) defined this cluster of risk factors, as the “metabolic syndrome” (Alberti & Zimmet, 1998; Cleeman et al., 2001; Detection, 2001; Zimmet, Alberti, & Shaw, 2005). The WHO briefly defined, “metabolic syndrome” as: insulin resistance and/or impaired fasting glucose (110-125 mmol/L) plus at least 2 of the following factors: obesity (body mass index (BMI) > 30 kg/m<sup>2</sup> and/or waist-to-hip ratio (WHR) > 0.9 for men and > 0.85 for women), dyslipidemia (HDL<sub>c</sub> < 0.35 for men and < 0.40 for women; and/or triglycerides > 150 mg/dL), hypertension (> 140/90 mmHg and/or antihypertensive medication) or microalbuminuria (albumin/creatinine ratio 25-250 mg/g). According to IDF, people suffer of “metabolic syndrome” if: central obesity with waist circumference > 94 cm for men and > 80 cm for women, plus at least 2 of the following factors: hypertriglycerimemia (> 150 mg/dL or specific treatment), low HDL cholesterol (< 40 mg/dL for men and < 50 mg/dL for women or specific treatment), hypertension (> 130/85 mmHg or treatment of previously diagnosed hypertension), impaired fasting glycemia (> 100 mg/dL or previously diagnosed T2D).

## 1.1 Management of diabetes

There are no therapies that can cure DM (Maiti, Jana, Das, & Ghosh, 2004) but there are many strategies available for the treatment such as: stimulation of endogenous insulin secretion, enhancement of insulin action at the target tissues, inhibition of dietary starch lipid degradation and pharmacological treatments with oral antidiabetic drugs (OADs) like biguanides and sulfonylureas (García-Pérez, Álvarez, Dilla, Gil-Guillén, & Orozco-Beltrán, 2013). However, these OADs can cause side effects such as major and minor hypoglycemia, gastrointestinal problems, peripheral edema, body weight gain, liver diseases and, over time, they lose their efficacy (García-Pérez et al., 2013).

The overall management of DM not only assures on achieving a normoglycemic state (HbA<sub>1c</sub> ≤ 6.5 mg/100 ml, fasting blood glucose ≤ 110 mg/100 ml) but also on reducing the risk for other metabolic diseases. To prevent metabolic diseases, visceral serum cholesterol should be ≤ 150 mg/100 ml, serum triglyceride ≤ 140 mg/ 100 ml, cholesterol low density lipoprotein (LDL<sub>c</sub>) ≤ 70mg/100 ml and high density lipoprotein (HDL) ≥ 60 mg/100 ml (Kaushik et al., 2010).

The limitation of these therapies has boosted 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. In the case of DM, it has been reported that numerous extracts obtained from plants can effectively reduce glycaemia (Gupta et al., 2005;

Lee & Sohn, 2009; Pushparaj, Tan, & Tan, 2000; Sohn et al., 2010). However little is known on the molecular mechanisms of protection activated when using such extracts.

In fact, the best way to “cure” T2D is “not getting” T2D, and this may be achieved by living an active life, practicing physical activity, reducing caloric intake and eating healthy food. Physical activity is negatively associated with risk factors for CVD, such as waist circumference, BMI, WHR and triglycerides and is positively associated with HDL (Aadahl, Kjaer, & Jorgensen, 2007). Nutritional guidelines include reduction of saturated fat and *trans* fatty acids, cholesterol <200 mg and the intake of n-3 polyunsaturated fatty acids from fish or seeds oils (Expert Panel on detection, 2002). Carbohydrate intake should be limited (60 % of total calories or 50 % in the presence of high triglycerides or low HDLc) and the consume of carbohydrate sources with high-fiber content is preferential rather than refined carbohydrates sources (Wolfram, 2007). Limiting the sodium intake to < 1.5 g/d (sodium chloride < 3.8 g/d) and increasing potassium intake (approximately to 4.7 g/d) (Appel et al., 2006) significantly reduces blood pressure and thus reduces cardiovascular risk (Appel et al., 2006; Sacks et al., 2001). All these parameters should be taken into account to avoid T2D.

Diabetes can lead to long-term complications such as atherosclerosis, hypertension, hypertriglyceridemia, hypercholesterolemia, myocardial infarction, ischemic attacks, impotence (Stadler, Jenei, von Bölcsházy, Somogyi, & Jakus, 2003), retinopathy (blindness), nephropathy (kidney damage), neuropathy (nerve damage) and diabetic foot (Islam, 2011). Diabetic patients possess a higher risk of death, together with lower survival rates and lower life expectancy than non-diabetic adults (Gu, Cowie, & Harris, 1998). Amongst all the comorbidities associated with T2D, the importance of CVD in diabetic individuals has been highlighted in recent years since CVD accounts for up to 80% of the death in T2D patients (Haffner, Lehto, Rönnemaa, Pyörälä, & Laakso, 1998). A population-based study showed that CVD mortality was 7.5-fold higher among T2DM individuals without a previous myocardial infarction than those without DM. The mortality was 3 times higher among diabetic patients who had suffer previous myocardial infarction than among nondiabetic individuals (Haffner et al., 1998). Interestingly, the risk of onset CVD due to diabetes was found to be greater in women than in men (Sowers, 1998).

The development of CVD in diabetic individuals can be related to several factors such as: enhanced platelet aggregation, relatively greater coagulation and decreased fibrinolytic activity, lipoprotein abnormalities, endothelial dysfunction, enhanced OS, vascular protein glycation and enhanced growth factor stimulation (Sowers, 1998).

## 1.2 Diabetes and oxidative stress

OS plays an important role in the development of vascular complications in diabetic individuals (Lipinski, 2001).

In diabetic individuals, free radicals are formed through several ways such as glucose oxidation, nonenzymatic glycation of proteins and the oxidative degradation of glycosylated proteins (Maritim, Sanders, & Watkins, 2003). The nonradical oxidants such as hydrogen peroxide, hypochlorous acid, singlet oxygen and radical oxygen species like superoxide anion and hydroxyl radicals, can attack the double bonds of unsaturated fatty acids promoting the formation of lipid peroxides (Lipinski, 2001).

This abnormal enhancement of free radicals and decline of antioxidant defense mechanisms lead to the damage of cellular organelles and enzymes, the increase in the lipids peroxidation and the increase of insulin resistance (Maritim et al., 2003). Thus, it is important to measure lipids peroxidation. The 2-ThioBarbituric acid reactive substances (TBARS) assay is a well-recognized method for quantifying the lipid peroxides (Devasagayam, Boloor, & Ramasarma, 2003). This method is based on the ability of malondialdehyde (MDA), which is one of the secondary products of lipid peroxidation, to react with thiobarbituric acid in acidic conditions at high temperatures to form a pink MDA-(TBA) complex (Sochor et al., 2012).

Protein oxidation originates carbonyl groups and their level in tissues and plasma is a stable marker of oxidative stress (Odetti et al., 1999). When protein side chains (especially arginine, lysine, proline and threonine) are oxidized, carbonyl groups are produced. Usually the content of these carbonyl groups is used as marker of protein oxidation and carbonyl group accumulation has been observed in several diseases such as diabetes, arthritis and Alzheimer's (Dalle-Donne, Rossi, Giustarini, Milzani, & Colombo, 2003).

The study of OS biomarkers like superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, lipid peroxidation, nonenzymatic glycosylated proteins and vitamins are essential in way to explore the mechanisms by which the increase of free radicals accelerates the development of diabetic complications (Maritim et al., 2003).

### **1.3 Diabetes and myocardial substrate metabolism**

The heart is known to be a pump that converts the chemical energy into mechanical work. To support its contractile activity, the heart requires a continuous supply of energy. To satisfy its energy requirements, the heart is capable of consuming a variety of exogenous substrates such as lactate, ketone bodies, glucose and fatty acids (Figure 1.3.1.). Mitochondrial oxidative phosphorylation is fueled with energy from electrons that are transferred from carbon fuels by dehydrogenation reactions that generate NADH and FADH<sub>2</sub> produced primarily in the fatty acid  $\beta$ -oxidation pathway, the citric acid cycle (CAC), from the pyruvate dehydrogenase reaction and glycolysis (William C Stanley et al., 2005). Nevertheless, glucose is a well-known reliable substrate, especially when the heart is subjected to unfavorable conditions (Marco G Alves, Oliveira, & Carvalho, 2011).

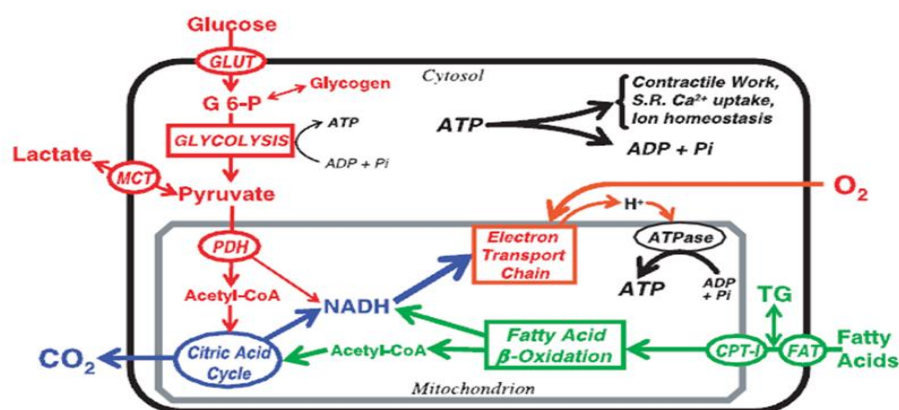


Figure 1.3.1. The myocardial substrate metabolism, pathways and regulatory points. The heart contractile force is regulated by the  $\text{Ca}^{2+}$  ions that enter the myocardial cells via  $\text{Ca}^{2+}$  channels triggering  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum, which allows contraction. Energy for contraction comes from the hydrolysis of ATP. The NADH and  $\text{FADH}_2$  generated by either the dehydrogenases of glycolysis, the oxidation of lactate and pyruvate and fatty acid  $\beta$ -oxidation, or the citric acid cycle deliver of electrons to the electron transport chain, resulting in ATP formation by oxidative phosphorylation. Lactate transport across the cardiac sarcolemma is facilitated by the monocarboxylic acid transporters (William C Stanley, Recchia, & Lopaschuk, 2005).

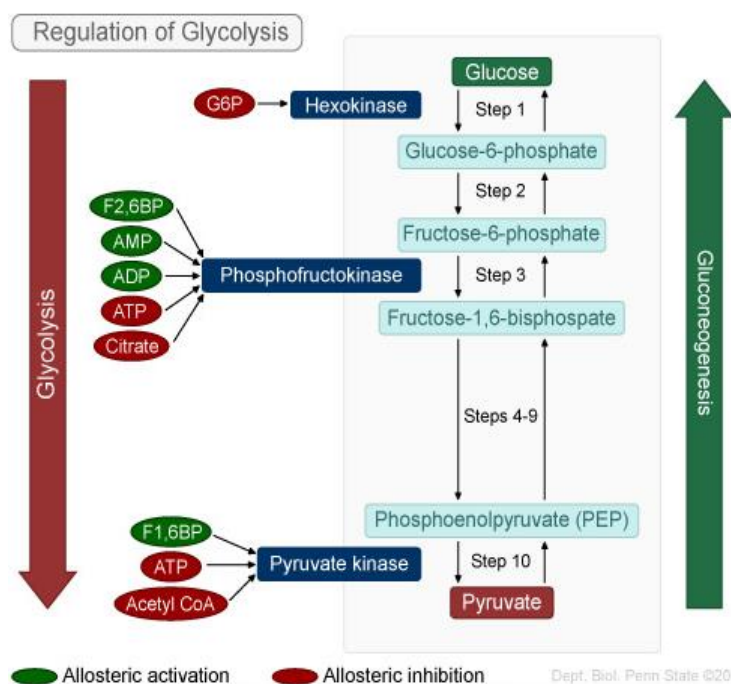
Intense exercise promotes a large increase in cardiac power but, in healthy heart, ATP content remains constant because the rate of oxidative phosphorylation is linked to the rate of ATP hydrolysis (Balaban, 1990). Indeed, the oxidative phosphorylation provides the greatest percentage (~95%) of ATP formation in the heart in normal healthy conditions. The remaining ATP comes from glycolysis and guanosine triphosphate formation in the CAC. The NADH and  $\text{FADH}_2$  formed in glycolysis, fatty acid oxidation, and the CAC are energy-rich molecules because each contains a pair of electrons having a high transfer potential. When these electrons are used to reduce molecular oxygen to water, a large amount of free energy is liberated, which can be used to generate ATP. In oxidative phosphorylation, ATP is formed as a result of the transfer of electrons from NADH or  $\text{FADH}_2$  to  $\text{O}_2$  by a series of electron carriers (Figure 1.3.1).

The heart metabolic machinery responsible for generating large amounts of ATP can be turned on or off by allosteric modification of regulatory enzymes, translocations of proteins to their site of function or changing the concentration of stimulatory or inhibitory metabolites. These mechanisms are responsible for the rapid adaptation to stress situations such as exercise, ischemia and metabolic diseases. As discussed earlier, in these conditions, glucose becomes the most reliable substrate for the heart maintenance of the contractile activity. The overall rate of glucose uptake is limited since the rate of glucose transport is slower than its phosphorylation (Depre et al., 1998). Thus, inside of cardiomyocytes the concentration of free glucose is lower than in blood, so that a gradient that favor the glucose entry is maintaining (Fischer, Becker, & Löken, 1999).

In glycolysis, the six-carbon sugar glucose is oxidized and split to create two molecules of pyruvate and two ATP are generated by each molecule of glucose. Products resulting of glycolysis, like pyruvate and NADH, are transported to mitochondria to generate  $\text{NAD}^+$  and  $\text{CO}_2$

or go to cytosol where they are converted to lactate and  $\text{NAD}^+$ . The plasma membrane of cells possesses a set of transporters that allow the movement of glucose either into or out of cells (Gould & Holman, 1993). The rate of entry of glucose into a cell is limited by the number of glucose transporters on the cell surface and the affinity of the transporters for glucose.

Due to insulin stimulation, occurs the translocation of glucose transporters from intracellular vesicles to sarcolemma membrane which increases the rate of glucose uptake and the ability of membrane to transport glucose (William C Stanley et al., 2005). When glucose transport is stimulated, hexokinase phosphorylate glucose originating glucose-6-phosphate (Fig.1.3.2). In mammalian cells the rate of formation of glucose-6-phosphate is reduced by the regulation of breakdown of glycogen. In addition to originate pyruvate, glucose-6-phosphate can be used to synthesize ribose for DNA and RNA nucleotides.



**Figure 1.3.2. Regulation of glycolysis.** The glycolytic pathway is shown on the left. The enzymes hexokinase, phosphofruktokinase and pyruvate kinase catalyze three important exergonic steps. For each of these pathways, the allosteric activators (labeled in green) and allosteric inhibitors (labeled in red) are indicated. Gluconeogenesis (shown on the right) is the reverse of glycolysis, with the exception of steps 1, 3, and 10 and the enzymes that catalyze these steps. (<https://wikispaces.psu.edu/download/attachments/46924785/image-1.jpg>)

The reaction catalyzed by phosphofruktokinase (PFK) is usually described as the most important regulatory step in glycolysis. PFK is regulated by the cell fraction of the adenosine nucleotides that contains high-energy bonds (energy charge of cell) thus, AMP and fructose-2,6-bisphosphate are activators of PFK. PFK 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 & Newsholme, 1965).

The myocardium becomes a lactate producer only when glycolysis is accelerated in face of impaired oxidation of pyruvate, such as in ischemia or poorly controlled diabetes (William C Stanley et al., 2005). When the heart is perfused, then pyruvate becomes the main substrate (Marco G Alves et al., 2011).

The three main fates to pyruvate formed from glycolysis are: 1) conversion to lactate, 2) carboxylation to oxaloacetate/malate or 3) decarboxylation to acetyl-CoA. In this system, the pyruvate kinase (PK) and the lactate dehydrogenase (LDH) play key roles. The PK catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP, yielding one molecule of pyruvate and one molecule of ATP. It is inhibited by acetyl-CoA, ATP and allosterically by fatty acids serving as an indicator that alternative energy sources are available for the cell. The activity of PK is increased when glycolysis is activated. In addition to carbon entry into the CAC via acetyl-CoA, carbon entry can also occur via so-called anapleurotic pathways (Lloyd, Brocks, & Chatham, 2003). Anapleurotic reaction is a metabolic reaction that replenishes intermediates of pathways. Due to carboxylation to malate or oxaloacetate (an “anaplerotic” reaction), pyruvate enters in CAC (Lloyd et al., 2003). The transamination of glutamate to  $\alpha$ -ketoglutarate and the formation of succinyl-CoA from propionyl-CoA are examples of others anaplerotic reactions (Lloyd et al., 2003).

The formation of CAC intermediate as  $\alpha$ -ketoglutarate and alanine by transamination of glutamate is also an important contribution of pyruvate to anaplerosis (William C Stanley et al., 2005). In cases where glycolysis is accelerated like hyperglycemia, the rate of alanine output is usually unaffected (William C Stanley et al., 2005).

In addition to being oxidized, pyruvate can also be converted to alanine via alanine aminotransferase and to lactate via LDH (Lloyd et al., 2003).

## 2 Tea

Tea is, next to water, one of the most consumed beverages in the world (Cheng, 2006). This beverage popularity is mainly associated with its taste and aroma, relative low retail prices, stimulating effects and known and possible health benefits (Baptista, da P Tavares, & Carvalho, 1999; Baptista, Tavares, & Carvalho, 1998).

Tea has a long history as a folk remedy, but his beneficial properties have mainly been elucidated in the past two decades (Anderson & Polansky, 2002). Tea is one of the oldest known medicine and has been used in China for the last 5000 years for its detoxifying properties as the elimination of alcohol and toxins, to improve blood circulation and urine flow, to relieve joint pains and to improve disease resistance (Balentine, Wiseman, & Bouwens, 1997).

Tea is an infusion prepared with the leaves of the plant *Camellia sinensis* (L.) from the Theaceae family (López & Calvo, 2011). There are two main varieties of tea plants: *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*. The *C. sinensis* var. *sinensis* is a small leaved, bushlike plant indigenous from China, which is widespread through all the Southeast Asia more adapt to a cold climate, and *C. sinensis* var. *assamica*, that is a large-leaved tree found in the Assam region, India, which is more adapt to semitropical climates (de Mejia, Ramirez-Mares, & Puangpraphant, 2009).

Tea was introduce to Europe from China by the Dutch and the Portuguese merchants (Hollman, Hertog, & Katan, 1996). The origins of modern tea industry happened between 1818-1834 in India through the importation of the var. *sinensis* and the discovery of native var. *assamica* (Harbowy, Balentine, Davies, & Cai, 1997). With the advances in technology and in the “know how”, new plantations were made across tropical areas like Africa, South America, Russia (Georgia) (Eden, 1976) and also in the island of S. Miguel, Azores Archipelago (Portugal) (Baptista et al., 1999).

### 2.1 Types of Tea

Tea diversity is due to the botanical variety, geographical origin (de Mejia et al., 2009) starting material, harvesting and processing method. The basic differentiation of tea, is related with the degree of “fermentation”: as white tea (WTEA) and green tea (GT) (not fermented), oolong (semi-fermented), and black tea (completely fermented) (M. P. Almajano, Carbo, Jiménez, & Gordon, 2008). The level of fermentation influences the composition (phenolic profile) and organoleptic properties (appearances and taste) of the tea (Moderno, Carvalho, & Silva, 2009). However this process is wrongfully defined as “fermentation”, the more correct term is oxidation (Bartlett, 2004), a reaction which is catalyzed by polyphenol oxidase (PPO).

There are two seasons for tea harvest: the “First Flush” in early spring and the “second flush” in summer. Once the tea leaves (leaf buds or tips, for WTEA) are picked, if they are not dried afterward, they turn progressively darker because chlorophylls break down and tannins are released. The oxidation process can, and is, interrupted by tea industry at a predetermined stage by heating, inhibiting PPO. This enzyme converts catechins (Cs) to theaflavins and their polymers, thearubigins, mainly present in fermented teas as oolong and black tea (Subramanian, Venkatesh, Ganguli, & Sinkar, 1999).

### 2.1.1 White tea

WTEA is prepared from very young tea leaves or leaf buds covered with tiny, silvery hairs, which are picked up before fully opened only once a year in the early spring (Espinosa et al., 2012). WTEA is steamed and dried quickly after harvest, preventing whitering and inactivating the PPO thus becoming the least processed of all teas (Mao et al., 2010).

## 2.2 Chemical composition

The manufacturing method influences the overall chemical composition of the tea, that is very complex and includes proteins, chlorophyll, organic and amino acids, polysaccharides, vitamins, carbohydrates, lignins, methylxanthines (caffeine, theophylline and theobromine), minerals and trace elements, volatile oils and flavonoids (Moderno et al., 2009; Seeram et al., 2006). Flavonoids are phenolic compounds that have unique biological properties that may be responsible for many health benefits attributed to tea (Rietveld & Wiseman, 2003). There are over 4000 different flavonoids described in the literature, and they are categorized in flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavonoids (Firenzuoli, Gori, Crupi, & Neri, 2004). The major class of phenolic compounds present in the tea leaves are catechins (Cs) (also known as flavan-3-ols), they represent 30-40 % of their dry weight (Karakaya & Kavas, 1999; Nihal, Ahmad, Mukhtar, & Wood, 2005; Wheeler & Wheeler, 2004). The main Cs of WTEA are: (-)-epicatechin (EC), (-)-epigallocatechin (EGC), defined as flavanol monomers, (-)-epicatechin 3-gallate (ECG), (-)-epigallocatechin 3-gallate (EGCG), which are known as flavanol gallates (Figure. 2.2.1) (Hara, Luo, Wickremasinghe, & Yamanishi, 1995a). The most abundant catechin in tea leaves is EGCG, 50-80% of total catechins, and is thought to exert the beneficial health effect ascribed to tea (Khan & Mukhtar, 2007; Ortsater, Grankvist, Wolfram, Kuehn, & Sjöholm, 2012)

Quercetin, kaempferol, myricetin, rutin and their glycosides are the main flavonols of tea (C. Dufresne & Farnworth, 2000; C. J. Dufresne & Farnworth, 2001). Also methylxanthines are present in tea, 2-4% as caffeine and small amounts of theophylline and theobromine (Hara et al., 1995a). From the amino acids present in tea, theanine is specific of the tea plant,

accounting for 50 % of the total amino acid content (C. Dufresne & Farnworth, 2000). Tea also contains carbohydrates, vitamins such as E, K, A, B and C (C. Dufresne & Farnworth, 2000). Large amounts of potassium, manganese and fluoride ions are provided by tea (Hara, Luo, Wickremasinghe, & Yamanishi, 1995b).

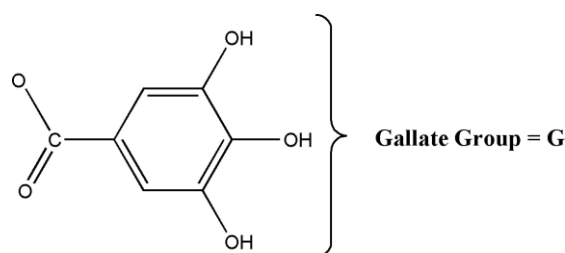
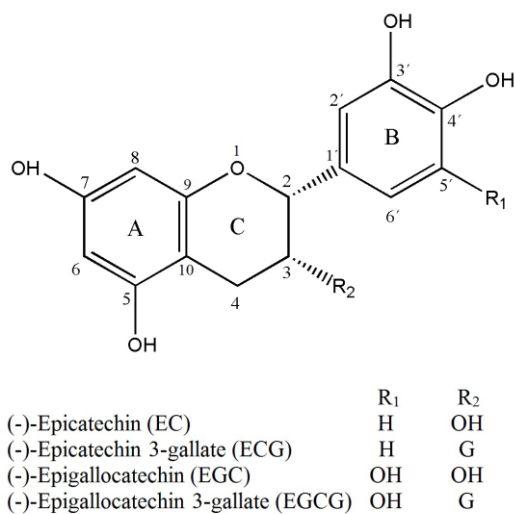


Figure 2.2.1. Main chemical structures of tea catechins (Dias et al.).

## 2.3 Metabolism, bioavailability and elimination of Catechins

Studies on bioavailability of phenolic compounds and their body distributions have been carried using sensitive and accurate methods to analyze metabolites in biological fluids and tissues (Hirayama, Takagi, Hukumoto, & Katoh, 1997; Hollman et al., 1996). The potential health effects of Cs depend on the amount ingested and on their bioavailability. (Okushio, Matsumoto, Korhi, & Susuki, 1996) identified the four major Cs (EC, ECG, EGC and EGCG) in the portal vein, following the oral administration, indicating that tea Cs are absorbed intestinally.

In a human study, (C. S. Yang, Lee, & Chen, 1999) reported that saliva possess a catechin esterase activity, suggesting that EGCG may be degalloylated in the mouth and esophagus. Furthermore Cs have been identified to suffer glucuronidation, sulfation, and O-methylation reaction by the enzymes UDP-glucuronosyltransferase (UGT), phenolsulfotransferase (PST) and catechol-O-methyltransferase (COMT) respectively, and also ring fission metabolism. The metabolic fate of tea constituents depends on their structure. Studies with radioactively

labeled Cs in humans shown that they are efficiently metabolized (Hollman et al., 1996). A study on the activity of conjugative enzymes in rat tissue found the highest UGT activity in the small's and large's intestine mucosa, the PST was in the liver and COMT was in the liver but also in the kidney (Piskula & Terao, 1998).

Polyphenols have shown a strong affinity for proteins, particularly when proteins have a high proline content such as casein, gelatin and salivary proteins (C. Dufresne & Farnworth, 2000). This protein-binding capacity may reduce the digestibility of alimentary proteins and rise fecal nitrogen excretion in humans, according to results observed in herbivorous animals (Hollman et al., 1996). Tea polyphenols had also demonstrated a strong interaction with transition metals and formed insoluble complexes with iron which reduce the bioavailability of non-heme iron. This bind inhibits iron gastrointestinal adsorption (Bravo, 1998; Hollman et al., 1996; Hurrell, Reddy, & Cook, 1999). Besides, L-ascorbic acid inhibits the formation of these complexes. A poor iron intake can result in increased chances of anemia. So, vegetarians may be advised of consuming tea brew during meals, because iron from plant is not available and the tea binding action may further reduce iron bioavailability. The absorption inhibition of zinc has been observed in rats, but copper results are not clear (C. J. Dufresne & Farnworth, 2001). Polyphenols can also affect the bioavailability of sodium and aluminum but not of calcium, magnesium or manganese (Bravo, 1998).

## **2.4 Antioxidant potential and health benefits of white tea**

In the last few years, antioxidant components have aroused great interest such as phenolic compounds from plant because of their ability to reduce the harmful effects of reactive oxygen and nitrogen species (RONS) on several biologic and pathologic processes (Alarcón, Campos, Edwards, Lissi, & López-Alarcón, 2008). The majority of the living organisms have an efficient protective mechanism against the excess production of RONS through enzymatic or non-enzymatic via. Yet there are several external factors (diet, alcohol, smoke, drugs, among others) and internal factors (such as aging) that decreases the efficiency of these endogenous antioxidant defenses, creating an impairment in the redox equilibrium of a healthy condition (Rietveld & Wiseman, 2003; Willett, 1994). Long exposure to RONS can damage DNA, lipidic membranes, lipoproteins, and structural and functional proteins (Halliwell, 1997; Sohal & Weindruch, 1996). RONS have been related with the development of several chronic diseases such as: CVD, DM, chronic inflammation, neurodegenerative disorders, and some types of cancer (Valko et al., 2007; Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006)

Several studies have reported that tea (poly)phenolic substances exert beneficial effects not only by scavenging RONS but also as metal chelators, preventing LDL oxidation, DNA strand scission and enhancing immune function (J. Cao, Xu, Chen, & Klaunig, 1996; Costa et al., 2009; Fattouch et al., 2007; Fiorentino et al., 2008; Giada & Filho, 2006; Magalhães et al., 2009; Oliveira et al., 2007; Silva, Valentão, Seabra, & Andrade, 2008). A number of structures

are reported to be important for this antioxidant activity of tea polyphenols (Figure. 2.2.1). One is 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, Balentine, & Frei, 1997); another is 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, Miller, & Paganga, 1996).

Studies in humans apparently demonstrate that the moderate consumption of GT and/or black tea (1-6 cups/day) significantly augmented the plasma antioxidant capacity after 1 hour (Rietveld & Wiseman, 2003).

The antioxidant power of Cs determined by the diphenylpicrylhydrazyl (DPPH) method was found to be: EGCG > ECG > EGC > EC > C; (Katalinic, Milos, Kulisic, & Jukic, 2006; Łuczaj & Skrzydlewska, 2005).

## 2.5 Influence of tea on diseases

Several scientific reports have shown that tea ingestion brings benefit to health and may play a role in the prevention of chronic diseases, such as CVD (Y. Abe et al., 1995; Cheng, 2000; Curin & Andriantsitohaina, 2005; Hodgson, Burke, & Puddey, 2005; Kono, Shinchi, Ikeda, Yanai, & Imanishi, 1992; Tokunaga et al., 2002), anti-inflammatory (Alschuler, 1998), immune response (Bhattacharyya, Mandal, Lahiry, Sa, & Das, 2004; Hu, Toda, Okubo, Hara, & Shimamura, 1992), anti-carcinogenic effect (Mukhtar, Katiyar, & Agarwal, 1994), anti-mutagenic (Santana-Rios et al., 2001; C. S. Yang, Chung, Yang, Chhabra, & Lee, 2000), anti-diabetic effect (Anderson & Polansky, 2002), longevity (Sadakata, Fukao, & Hisamichi, 1992), anti-neurodegenerative disorders (M. Almajano, Vila, & Ginés, 2011; Barranco Quintana, Allam, Del Castillo, & Navajas, 2009; Checkoway et al., 2002; Commenges et al., 2000; Tan et al., 2008) and antimicrobial (M. P. Almajano et al., 2008; Nakayama et al., 1993; Taguri, Tanaka, & Kouno, 2004; Weber, Ruzindana-Umunyana, Imbeault, & Sircar, 2003). EGCG, the most abundant phenolic compound in non-fermented teas, such as WTEA and GT, has been reported to possess vasculoprotective effect (Potenza et al., 2007; Wolfram, 2007).

## 2.6 Antidiabetic potential

There are several reports showing that plant extracts are efficient in reducing glycemia, lowering the side effects and costs presented by traditional OADs (Gupta et al., 2005; Lee & Sohn, 2009; Pushparaj et al., 2000; Sohn et al., 2010). There are some evidence that tea is an hypoglycemic agent (Todd MacKenzie, Lisa Leary, & W Blair Brooks, 2007). However, the exact mechanism by which tea ameliorates DM has not been elucidated yet. All the studies

suggest that tea polyphenols do not increase insulin secretion, but rather decrease insulin resistance and ameliorates insulin sensitivity (Islam, 2011).

It is well known that hyperglycemia increases the formation of RONS and decreases antioxidant endogenous mechanisms (Rahimi, Nikfar, Larijani, & Abdollahi, 2005). OS triggered by hyperglycemia seem to be one the major causes for post DM complications (Valko et al., 2007). In both T1D and T2D there is an increase of OS (Naziroğlu & Butterworth, 2005). It was shown that cytokine-induced  $\beta$ -cell damage was reduced by EGCG *in vitro* preventing the reduction of islet mass induced by treatment with streptozotocin (STZ) (Song, Hur, & Han, 2003). Yet in another study where STZ was co-injected with EGCG, it was unclear if the protective effect observed was due to direct inhibition of STZ by EGCG (I. Abe, Kashiwagi, & Noguchi, 2000). An study by (Roghani & Baluchnejadmojarad, 2010) reported that EGCG supplementation reduced serum glucose, total cholesterol and triglycerides and LDL<sub>c</sub> in STZ-induced diabetic rats. The effect of a single catechin from tea, EGCG, can be different from a whole crude tea extract.

## 2.7 Anti-CVD

In a cohort study involving 40530 Japanese adults, the consumption of 5 or more cups of GT per day was found to significantly reduce mortality due to all causes (-16 %) and CVD (-26 %) compared to subjects consuming less than 1 cup per day (Kuriyama et al., 2006). Interestingly, the beneficial effect of GT on CVD mortality increased according to the overall consumption evidencing that GT may indeed have a cardioprotective effect (Wolfram, 2007). Besides, it is well known that hypertension is the major risk factor for stroke and CVD. In a cross-sectional study by (Y.-C. Yang, Lu, Wu, Wu, & Chang, 2004), the role of habitual consumption of GT was observed in 1507 Chinese subjects. Individuals ingesting 120-599 ml of GT per day for at least one year, presented reduced risk of developing hypertension in 46 % when comparing with those consuming less than 120 mL per day. Noteworthy, those who consumed more than 600 mL per day reduce their risk by 65 %.

In another study by (Sasazuki et al., 2000), it was reported an inverse association between GT consumption and coronary atherosclerosis. A subgroup of T2D patient without dietary or drug treatment presented a reduced risk of stenosis in 50% for subjects consuming 2-3 cup a day and 60% for those consuming 4 or more cups, in comparison to those consuming 1 cup or less per day.

In a retrospective cohort study among 17413 Japanese adults, the risk of developing DM drop 33% in subjects consuming 6 or more cups of GT per day, in comparison to those consuming less than 1 cup per week (Iso, Date, Wakai, Fukui, & Tamakoshi, 2006). Interestingly, in women, there is a strong dose-response relationship and it was observe for subjects consuming 1-6 cups per week, 1-2, 3-5, and 6 or more cups per day a reduction of 21%, 34%, 39% and 51% respectively (Wolfram, 2007). These studies provide clear evidence that the

habitual consumption of tea is inversely associated with CVD mortality, the risk of developing hypertension and DM.

In an open, uncontrolled study by (W. Kim et al., 2006) on 20 chronic smokers, the effect of GT consumption on flow-mediated dilation was investigated. During 2 week, every day, they consumed 8 g of powdered GT. Tea consumption significantly enhanced the flow-mediated dilation and the number of circulating endothelial progenitor cells. The authors suggested that GT consumption may prevent future cardiovascular events in smokers. Similar results were obtained in a cross-over study by (Nagaya et al., 2004) where the forearm blood flow was enhanced and the urinary concentration of an oxidative stress marker, 8-iso-prostaglandin-F<sub>2</sub>α, was reduced by the consumption of 400 mL GT (~247 mg EGCG in ~692 mg total catechins per day). (Widlansky et al., 2007) performed a randomized, double-blind, placebo-controlled, cross-over study in 42 subjects with endothelial dysfunction and investigated the effects of supplementation with 300 mg EGCG daily for 2 weeks on brachial artery flow-mediated dilation. The results showed that EGCG acutely improved brachial artery flow-mediated dilation and the changes in vascular function are paralleled to the changes in EGCG plasma concentration. Later, an intervention study by (Widlansky et al., 2007) was the first to report a cardiovascular benefit from a single tea catechin.

In a randomized, controlled study in a parallel, open-label design, (Hakim et al., 2003) it was studied the antioxidant effect of decaffeinated GT or black tea consumption (4 cup per day) during 4 months in 133 smokers. When compared to water and black tea, GT consumption (~144 mg EGCG in ~294 mg total Cs per day) decreased urinary 8-hydroxydeoxyguanosin, a marker of DNA oxidative damage. Analogous results were obtained in a randomized, double-blinded, placebo-controlled trial by (Luo et al., 2006) with subjects with a high risk for hepatocellular carcinoma. The consumption of 500 or 1000 mg of GT Cs per day, for the period of 3 months, resulted in a significant decrease of the levels of 8-hydroxydeoxyguanosin compared to placebo. Besides, GT consumption increased the antioxidant capacity and decreased plasma peroxides in plasma and also reduced oxidative damage and glutathione peroxidase activity in lymphocytes (Erba et al., 2005).

The acute effects of GT on glucose tolerance were investigated in young healthy Japanese volunteers (Tsuneki et al., 2004). It was observed that the consumption of 1.5 g of GT extract, 20 min before an oral glucose load significantly reduced plasma glucose levels during the glucose tolerance test.

Hase et al. (2001) have reported that the consumption of GT Cs (300 mg EGCG in 480 mg total Cs) for 3 months, reduced glucose and insulin levels in healthy, slightly overweight, Japanese subjects. These results suggested that chronic consumption of GT Cs improves insulin sensitivity, but it also caused weight loss, which as a consequence may have decreased the levels of glucose and insulin.

Ryu et al. (2006) have studied the effect of the daily consumption of 900 mL water containing 9 g of GT, over a month period, in 55 Korean subjects with T2D. A trend towards reduce fasting glucose was observed but no other parameter was changed.

A study by H. L. Li et al. (2006) investigated the effects of EGG on cardiac hypertrophy *in vitro* and *in vivo*. In rats, the cardiac hypertrophy was induced by pressure overload due to constriction of the abdominal aorta; afterward they received oral EGCG (50 mg/kg) for 3 weeks. The EGCG treatment prevented the overload-induced cardiac hypertrophy, the increase of systolic blood pressure and the decrease in fractional shortening. EGCG reduced the generation of ROS and the expression of NADPH oxidase due to angiotensin II and the pressure overload. EGCG inhibiting angiotensin II, induced the activation of nuclear factor- $\kappa$ B and activator protein-1. Hotta et al. (2006) observed another cardiovascular benefit of EGCG in guinea pig hearts. The left ventricular pressure was increased as well nitric oxide (NO) and the calcium content of the heart, when EGCG was added to the perfusion medium (10 and 100  $\mu$ M). Therefore, EGCG had a positive inotropic effect without accompanying positive chronotropic effects in an NO-dependent manner.

The effect of GT in rats with STZ-induced DM has already been documented (P. V. A. Babu, K. E. Sabitha, & C. S. Shyamaladevi, 2006c). These authors observed that oral intake of GT extract (300 mg/kg for 4 weeks) reduced lipid peroxides and the activity of antioxidant enzymes, while increased the content of glutathione in the heart and aorta of diabetic rats. In other study, the same group found reduced blood glucose, lipid peroxides, triglycerides and protein glycation in the heart of diabetic rats (P. V. A. Babu, K. E. Sabitha, & C. S. Shyamaladevi, 2006a). Furthermore, the rise in cardiac calcium and sodium concentrations were blunted by GT extract due to increased calcium-ATPase and sodium/potassium-ATPase activities in diabetic rats. In another study by P. Babu, K. Sabitha, and C. Shyamaladevi (2006), GT extract caused recovery of body weight and heart weight/body weight ratio, reduced blood glucose, serum cholesterol, triglycerides, free fatty acid, and LDL<sub>c</sub> and increased HDL<sub>c</sub>. In myocardium, GT extracts decreased cholesterol, triglycerides and free fatty acid and also the activity of lipoprotein lipase. Also, GT extract reduced systolic blood pressure, blood glucose, serum lipid peroxides and elevated serum glutathione and vitamin C in another study from P. V. A. Babu, K. E. Sabitha, and C. S. Shyamaladevi (2006b), with the same experimental design. The accumulation of aortic collagen, extent of glycation, advanced glycation end-products (AGE) formation and cross-linking of collagen were reduced by the GT extract. Similar results were reported, in other study, where GT extract reduced the rise in blood glucose, glycated hemoglobin and systolic blood pressure in diabetic rats (Babu, Sabitha, Srinivasan, & Shyamaladevi, 2007). It also prevented diabetes-induced increase in lactate dehydrogenase, aspartate transaminase and creatine kinase activities in serum and Maillard-type fluorescence and collagen cross-linking in myocardium.

The effect of EGCG (25, 50, 100 mg/kg for 50 days) was investigated in rats with STZ-induced diabetes and subtotal nephrectomy (Yamabe, Yokozawa, Oya, & Kim, 2006). EGCG reduced lipid peroxidation, proteinuria, hyperglycemia and the renal AGE accumulation in the kidney cortex.

Other study reported that GT Cs inhibited the production of superoxides, lipid peroxides, and oxidized protein in the STZ-induced diabetic rats kidney (Choi et al., 2004). Furthermore, it

was suggested that GT Cs decreased inflammatory response due to reduced leukotriene B<sub>4</sub> production in the leukocytes. Mustata et al. (2005) studied the effects of GT consumption during 12 months on intra- and extracellular OS and glucotoxicity in STZ-induced diabetic rats, and found that it reduces the OS induced by DM. This effect was evaluated by the increase of erythrocyte glutathione content, as well as by the decrease of retinal superoxide formation, plasma peroxides and renal mitochondrial respiratory chain defects. However, tendon collagen glycoxidation and cross-linking were worsened, suggesting that GT may exert effects in the intra- and extracellular compartment. GT did not alter blood glucose and glycated hemoglobin.

Wu, Juan, Ho, Hsu, and Hwang (2004) investigated the effects of GT consumption on insulin sensitivity in rats. GT extract was administered via drinking water (~370 mg/kg for 4 months) with a regular diet, and rats displayed significantly decreased fasting plasma levels of glucose, insulin, free fatty acids and triglycerides. Plasma insulin level was lower in the GT consuming rats during an oral glucose tolerance test. However, the adipocytes isolated from these rats showed an enhanced capacity for glucose uptake and increased specific insulin binding than the adipocytes from the control group.

Li, Douglas, Maiyoh, Adeli, and Theriault (2006) investigated in fructose-induced insulin-resistant hamster model, the effects of a GT extract (300 mg/kg for 1 month) on the glucose and lipid metabolism, and found improvements in the oral glucose tolerance and reduced serum insulin levels. Furthermore, the protein expression of PPAR $\alpha$  and PPAR $\gamma$  was increased, suggesting that GT extract modified glucose and lipid metabolism partly via PPARs.

## **II . Aim of the present study**

Diabetes is a deleterious disease that causes severe damage to the cardiovascular system and tea, in this case WTEA, is known for its antioxidant power, higher than that of GT. The aim of the present study is to discover how diabetes affects the heart, and the possible protective effects of WTEA regular consumption in this condition.

The first objective was to evaluate the antioxidant state of the heart tissue, lipid peroxidation and protein oxidation of the STZ-induced diabetic rats and compare with the STZ-induced diabetic rats consuming WTEA, by using techniques such as FRAP assay, TBARS assay and the carbonyl group analysis. The second objective was to observe the metabolomics pool of the heart, studying the levels of glucose, lactate, alanine and acetate, the changes of key transporters such as GLUT1 and MCT4 and the expression and activity of LDH. To achieve this purpose we have resorted to NMR analysis, RT-PCR and LDH activity assay.

## **III . Materials and Methods**

### 3.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.), unless specifically stated.

### 3.2. White tea infusion

WTEA and GT samples were purchased on the Portuguese market. Samples were submitted to infusion (1 g/100 mL), for 3 min, according to the manufacturer's instructions. The resulting infusions were filtered with a sterile syringe filter of 0.2 µm cellulose acetate (VWR, Pennsylvania, U.S.A.).

### 3.3. Animal model and experiment design

In this study were used eighteen male Wistar rats, obtained from an accredited animal colony (Health Science Research Center, University of Beira Interior). They were maintained on *ad libitum* food in a room with constant temperature (20±2°C) and a 12 hour cycle of artificial light. Animals were randomly distributed into a control, a STZ-induced T2DM (STZ) and a STZ-induced T2DM with white tea consumption (STZ+Wtea) group. Animals from STZ and STZ+Wtea groups were injected with a low-dose of STZ to achieve a T2DM model, according to the method described by (Iwase et al., 1986). Briefly, two-days-old male Wistar rats were fasted for 8 hours. STZ and STZ+Wtea groups of animals were injected with STZ (40 mg/kg, i.p.) freshly diluted in citrate buffer (0.1, Na citrate, pH 4.5). The control group received only the vehicle solution in an equivalent volume. All groups were fed a standard chow diet (4RF21 certificate, Mucedola, Italy). The water from the STZ+Wtea group was replaced by WTEA after reaching 1 month of age, during 2 months. Animal's blood glucose levels were monitored every 6 days. Non-fasting glycemia was determined using a glucometer (One Touch Ultra Lifescan-Johnson, Milpitas, CA, U.S.A.). After treatment, animals were sacrificed by cervical dislocation. Heart was removed, weighed and stored at -80°C. 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 rules for the care and handling of laboratory animals (Directive 86/609/EEC).

### **3.4. Insulin and glucose tolerance tests**

At 3 months of age, animals were submitted to a glucose tolerance test, as previously described by (L Rato et al., 2013). Briefly, 14-18 hours before the test, the animals were deprived from food, kept in fast. An i.p. injection with 6 mL of glucose 30% (w/v) per kg of body weight was given to each animal. Blood samples for glucose measurement were obtained from the tail the moment before the glucose injection was given and also at 30, 60, 90 and 120 min. At the same age; the animals were subjected to an insulin resistance test, as described by Holmes and collaborators (Holmes et al., 2008). Briefly, an i.p. injection with 0.75 U insulin per kg of body weight was given to each animal. Blood samples for glucose measurement were obtained as previously mentioned.

### **3.5. Ferric reducing antioxidant power assay**

The ferric reducing antioxidant power (FRAP) of the heart samples was performed according to the colorimetric method described by (Benzie & Strain, 1996). Briefly, heart tissue was homogenized in phosphate buffer (pH 7.4). Protein concentration was determined by the Bradford micro-assay using BSA as a standard. Working FRAP reagent was prepared by mixing acetate buffer (300 nM, pH 3.6), 2,4,6-Tripyridyl-s-Triazine (TPTZ) (10 mM in 40 mM HCL) and  $\text{FeCl}_3$  (20 mM) in a 10:1:1 ratio (v:v:v). 180  $\mu\text{L}$  of FRAP reagent were mixed with 5  $\mu\text{g}$  of tissue homogenate. The reduction of the  $\text{Fe}^{3+}$ -TPTZ complex to a colored  $\text{Fe}^{2+}$ -TPTZ complex by the samples was immediately monitored after adding the sample and 15 min later, by measuring the absorbance at 595 nm using an Anthos 2010 microplate reader (Biochrom, Berlin, Germany). Antioxidant potential of the samples was determined against standards of ascorbic acid, which were processed the same way as the samples. Absorbance results were corrected by using a blank, with  $\text{H}_2\text{O}$  instead of sample.

### **3.6. Thiobarbituric acid reactive species assay**

Thiobarbituric acid reactive species (TBARS) are formed as a byproduct of lipid peroxidation, which can be detected by the TBARS assay using thiobarbituric acid (TBA) as a reagent. Malondialdehyde (MDA) is a product of this peroxidation reaction that reacts with TBA in conditions of high temperatures and low pH, generating a pink colored complex, which absorbs at 532 nm (Ohkawa, Ohishi, & Yagi, 1979). Heart tissue was homogenized in phosphate buffer (pH 7.4) and protein concentration was determined by the Bradford method using BSA as a standard. TBARS assay was carried out by a standard method described by (Iqbal et al., 1996) with slight modifications. Briefly, 10  $\mu\text{g}$  of tissue homogenate, 0.01 mL Tris-HCL buffer (150 mM, pH 7.1), 0.01 mL ferrous sulphate (1.0 mM), 0.01 mL L-ascorbic acid

(1.5 mM) and 0.06 mL H<sub>2</sub>O were mixed in a reaction tube. This mixture was incubated at 37 °C for 15 min. The reaction was stopped by adding 0.1 mL trichloroacetic acid (10% w/v). Subsequently, 0.2 mL of TBA (0.375% w/v) were added and all samples were incubated for 15 min in boiling water (100 °C). Finally, samples were centrifuged at 1000.g for 10 min. The amount of MDA formed in each sample was estimated by measuring optical density at 532 nm using a UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan) against a blank. The results were expressed as nmol of TBARS/mg protein.

### **3.7. Analysis of carbonyl groups**

Carbonyl groups content was determined following the protocol by Robinson and collaborators (Robinson et al., 1999). Briefly, a determined volume of homogenized tissue containing 20 µg of protein was derivatized with DNPH. It was added 1 volume of SDS 12% and 2 volumes of 20mM of DNPH in 10% TFA to the samples, followed by 30 minutes incubation in dark. After this period, 1.5 volumes of Stop solution (2M of Tris and 18 % of B-mercaptoethanol) was added. A dilution was made in PBS and the final concentration was 0.001 µg/ul. A volume of 100 µl per sample was transfer by slot-blot to a hybond-PVDF membrane. The membrane was then block, during 90 minutes, with a 5% non-fat milk at room temperature, followed by an overnight incubation at 4 °C with Anti-DNP (1:10000, V0401; DakoCytomation). The membrane was incubated with goat anti-rabbit igG-peroxidase (1:5000; sc2007; Santa Cruz Biotechnologies, Hiedelberg, Germany) for 90 minutes. Membranes were reacted with ECF<sup>TM</sup> (GE, Healthcare), a fluorescent substrate for alkaline phosphatase-based detection, and read with the BioRad FX-Pro-plus (Bio-Rad, Hemel Hempstead, UK). Densities from each slot were obtained with BIO-PROFIL Bio-1D Software from Quantity One (Vilber Lourmat, Marne-la-Vallée, France) according to standard methods (Martinez-Picado, Savara, Sutton, & Richard, 1999). The band density attained was divided by the corresponding control band intensities and expressed in fold variation (induction/reduction) versus the control group.

### **3.8. Metabolites extraction for NMR analysis**

Metabolites were extracted using a methanol/chloroform/water strategy as previously described (Martineau, Tea, Loaec, Giraudeau, & Akoka, 2011). Briefly, the heart tissue was placed in conical tubes. 1 mL of chloroform was added, and the tubes were vigorously vortexed and sonicated. 1 mL of chloroform/water (1:1) was then added and the samples were vortexed and centrifuged at 1000 g (4°C) during 15 min for phase separation. The upper methanol-water phase containing the water soluble cellular metabolites was carefully separated and lyophilized overnight. For NMR analysis, the lyophilized samples were dissolved in D<sub>2</sub>O.

### 3.9. NMR spectroscopy

<sup>1</sup>H NMR spectroscopy of metabolites extracted from heart tissue was performed using standard methods previously described (M. G. Alves, Neuhaus-Oliveira, Moreira, Socorro, & Oliveira, 2013). Briefly, <sup>1</sup>H NMR spectra of the collected samples were acquired at 14.1 T, 25 °C, using Bruker Avance 600 MHz spectrometer equipped with a 5-mm QXI probe with a z-gradient (Bruker Biopsin, Karlsruhe, Germany). Solvent-suppressed <sup>1</sup>H NMR spectra were acquired with a sweep width of 6 KHz, using a delay of 14 s, a water presaturation of 3 s, a pulse angle of 45°, an acquisition time of 3.5 s and at least 128 scans. Before Fourier transform, each free induction decay was zero filled and multiplied by a 0.2 Hz Lorentzian. Sodium fumarate (final concentration of 2 mM) was used as an internal reference (singlet, 6.50 ppm) to quantify the metabolites in solution (multiplet,  $\delta$ , ppm): lactate (doublet, 1.33); alanine (doublet, 1.45); H1- $\alpha$  glucose (doublet, 5.22). The relative areas of <sup>1</sup>H NMR resonances were quantified using the curve-fitting routine supplied with the NUTSpro NMR spectral analysis program (Acorn NMR, Inc, Fremont, CA).

### 3.10. RT-PCR

Total RNA (RNAt) isolated from the heart tissue was extracted using TRIzol reagent according to the manufacturer's instructions, resuspended in 10-30  $\mu$ L or 500-600  $\mu$ L of RNase-free water and stored at -80°C. RNA concentration and absorbance ratios (A260/A280) were determined by spectrophotometry (Nanophotometer<sup>TM</sup>, Implen, Germany). The reverse transcriptase reaction was performed in a final volume of 20  $\mu$ L containing: 200 U of M-MLV RT, 250 ng of random hexamer primers and 0.5 mM of each dNTP. The reaction was carried out at 37°C for 60 min. The resulting cDNA was used to amplify B2M, LDHa, GLUT1, and MCT4 cDNA fragments with exon-exon spanning primer sets. Each PCR reaction contained 1  $\mu$ L of cDNA, 200 nM of each 5' and 3' oligonucleotide primers, 0.5 U of Taq polymerase, 2.5  $\mu$ L of taq polymerase 10x buffer, 1.5 mM of MgCl<sub>2</sub>, 1  $\mu$ L of dNTPs 10 mM and distilled water up to 25  $\mu$ L. Primer sequences, optimal annealing temperature, the number of cycles required for exponential amplification phase of fragments and fragment sizes are indicated in Table 1. B2M, LDHa, GLUT1, e MCT4 mRNA levels were normalized with 18S gene expression as internal control.

**Table 1.** Primer sequences, optimal annealing temperature, number of cycles required for exponential amplification phase of fragments and fragment sizes.

Gene	Primer Sequence (5'-3')	AT(°C)	Amplicon size (bp)	C
GLUT1	Sense: TCCATTCTCCGTTTCACAGC Antisense: CCGGTGTTATAGCCGAACTG	55	145	40
LDH	Sense: CGTCGTCCCCATCGTGAC Antisense: GGGCCCCGCGGTGATAATG	60	345	35
MCT4	Sense: ACACTTAGGAGACAACAC Antisense: GGCAATATAGGAGACTGG	47	132	37
β-2 Microglobulin	Sense: ATGAGTATGCCTGCCGTGTG Antisense:CAAACCTCCATGATGCTGCTTAC	58	92	30
18s	Sense: AAG ACG AAC CAG AGC GAA AG Antisense: GGC GGG TCA TGG GAA TAA	56	149	25

### 3.11. LDH enzymatic activity

The activity of LDH was spectrophotometrically assessed by determining the cleavage of the respective colorimetric substrate as previously described (L Rato et al., 2013). The LDH activity was determined using a commercial assay kit (Promega, Madison, WI, USA) and following the manufacturer's instructions. Briefly, enzymatic activity was calculated by measuring the shift on the absorbance (492 nm) of samples resulted from the conversion of a tetrazolium salt (INT) into a red formazan product as the amount of formazan formed is directly proportional to the activity of LDH on the samples. The method was calibrated with the LDH Positive Control induced in the assay kit. The attained activities were calculated using the molar absorptivity of formazan and expressed as fold variation versus the control condition.

### 3.12. Statistical analyses

The statistical significance of mRNA and protein expression, LDH activity and antioxidant parameters among the experimental groups was assessed by one-way ANOVA, followed by Dunn post-test. All experimental data are shown as mean ± SEM (n=6 for each condition). Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA). P<0.05 was considered significant

## **IV. Results**

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## 4.1. White tea possesses a higher antioxidant power than green tea

WTEA and GT antioxidant capacities were compared by the FRAP assay. According to the literature, WTEA is thought to possess greater antioxidant potential than GT. As expected, the FRAP value in WTEA was significantly higher than in GT (Figure 4.1.1.). The GT had a FRAP value of  $16 \pm 0.1 \mu\text{M}$  while the FRAP value to WTEA was  $25 \pm 0.2 \mu\text{M}$ .

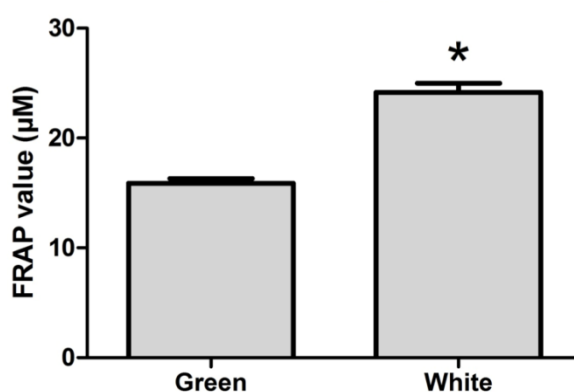


Figure 4.1.1. Antioxidant power of green and white tea measured by FRAP assay. FRAP value ( $\mu\text{mol/L}$ ) of antioxidant potential of green and white tea. Results are presented as mean  $\pm$  SEM ( $n=6$  for each condition). Significant results ( $p<0.05$ ) are as indicated: \* vs. green tea.

## 4.2. General characteristics of the STZ-induced diabetes model and effect of White tea consumption

After 60 days, the animals were weighted. The body weight of the STZ group ( $352 \pm 32 \text{ g}$ ) was similar to the control group ( $347 \pm 20 \text{ g}$ ) (Table 2). However, the rats from STZ group consumed more food ( $2730 \pm 39 \text{ g}$ ) than the rats from the control group ( $2384 \pm 17 \text{ g}$ ). As expected, rats of the STZ group ingested more water ( $3595 \pm 81 \text{ mL}$ ) than the control group ( $3227 \pm 56 \text{ mL}$ ). Finally, the STZ-treated group developed the general characteristics associated with T2DM. The average glycaemia in the rats from the STZ-treated groups was increased (STZ and STZ+Wtea:  $119 \pm 2 \text{ mg/dL}$  and  $117 \pm 2 \text{ mg/dL}$ , respectively) in comparison with glycaemia values determined for rats in the control group ( $90 \pm 1 \text{ mg/dL}$ ) (Table 2).

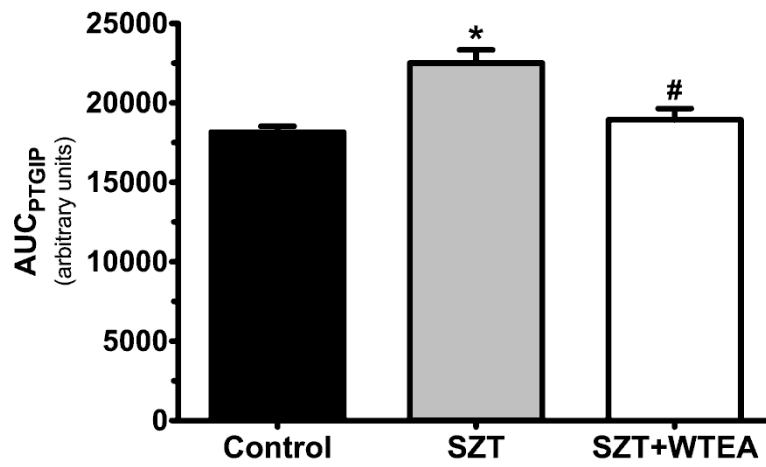
**Table 2.** Mean values of weight, blood glycaemia, water intake and food consumption in rats, after 60 days. Results are expressed as mean  $\pm$  SEM (n=6 for each condition). \* Significantly different relative to control group (p<0.05). # Significantly different relative to STZ group (p<0.05).

Factors	Control group	STZ group	STZ+Wtea group
Weight (g)	347 $\pm$ 20	352 $\pm$ 32	378 $\pm$ 32
Glycemia (mg/dL)	90 $\pm$ 1	119 $\pm$ 2*	117 $\pm$ 2*
Drink intake (mL)	3227 $\pm$ 56	3595 $\pm$ 81*	3143 $\pm$ 35 <sup>#</sup>
Food consumption (g)	2384 $\pm$ 17	2730 $\pm$ 39*	2920 $\pm$ 9 <sup>*#</sup>

The STZ-induced diabetic rats developed glucose intolerance. This can be concluded by the analysis of the area under the curve (AUC). AUC is defined by the integral between the function  $y=f(x)$  and the x axis. The AUC of the STZ-induced diabetic group (22508 arbitrary units) in the glucose tolerance test was significantly higher than the AUC detected in rats from the control group (18153 units) (Figure 4.2.1. A). The AUC in the STZ-induced diabetic rats consuming WTEA (18932 units) was significantly lower in comparison with the STZ-induced diabetic rats (Figure 4.2.1. A).

Besides glucose intolerance, insulin resistance was also determined in the STZ-treated animals. STZ-induced diabetic rats presented an AUC almost 2-folds higher than the rats from the control group which implies a higher insulin resistance in the STZ-induced diabetic rats, a main characteristic of T2DM (Figure 4.2.1 B). Noteworthy, the STZ+Wtea group scored a significantly lower AUC (9026 units) when compared to the STZ group in the insulin resistance test (Figure 4.2.1. B). Importantly, there were no significant differences between the STZ+WTEA group and the control group in both tests.

A



B

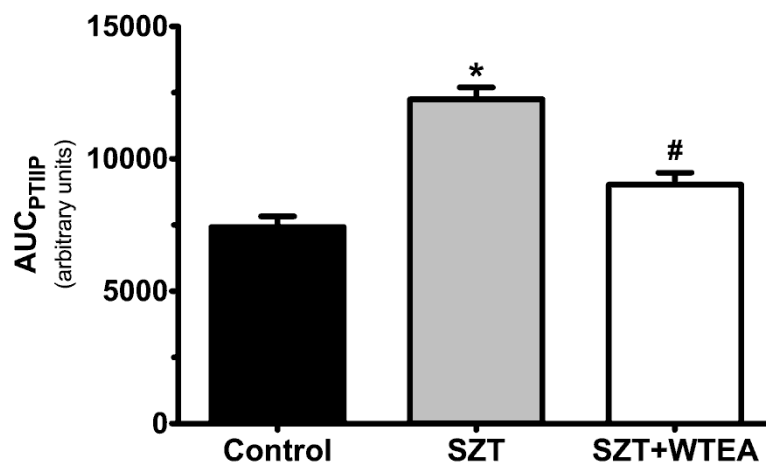


Figure 4.2.1. Effect of white tea consumption by STZ-induced T2DM rats in the glucose tolerance and insulin resistance test. Graph A: shows AUC of control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats consuming WTEA (STZ+WTEA), during the i.p. glucose tolerance test. Graph B shows the AUC blood glucose levels in control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic consuming WTEA (STZ+WTEA), measured during the i.p. insulin resistance test. Results are expressed as mean  $\pm$  SEM (n=6 for each condition). Significant results ( $p < 0.05$ ) are indicated as: \* vs. control; # vs. STZ group.

### 4.3. Heart tissue antioxidant potential of STZ-induced diabetic rats is increased by white tea ingestion

The heart tissue antioxidant capacity was determined by the FRAP assay. The FRAP assay measures the potential of an antioxidant to reduce ferric (III) to ferrous (II) in a redox-linked colorimetric reaction that involves single electron transfer (Benzie & Strain, 1996). The reducing capacity of a compound/extract and/or cell and tissues serves as a direct significant parameter of its potential antioxidant activity (FRAP value). The antioxidant potential of the heart tissue, as expected, was significantly reduced in STZ-induced diabetic rats. In fact, the FRAP value decreased from 2.5  $\mu\text{mol}$  antioxidant potential/mg of protein in the heart tissue from the control rats to 0.7  $\mu\text{mol}$  antioxidant potential/mg of protein in the heart tissue from STZ-induced diabetic rats (Figure 4.3.1.). Noteworthy, the antioxidant potential of the heart tissue from the STZ-induced diabetic rats that ingested WTEA is 2-fold higher than those of the heart tissue of STZ-induced diabetic rats that did not ingest WTEA (Figure 4.3.1.). Still, the antioxidant capacity of the STZ-induced diabetic rats that consumed WTEA was significantly different from the control group.

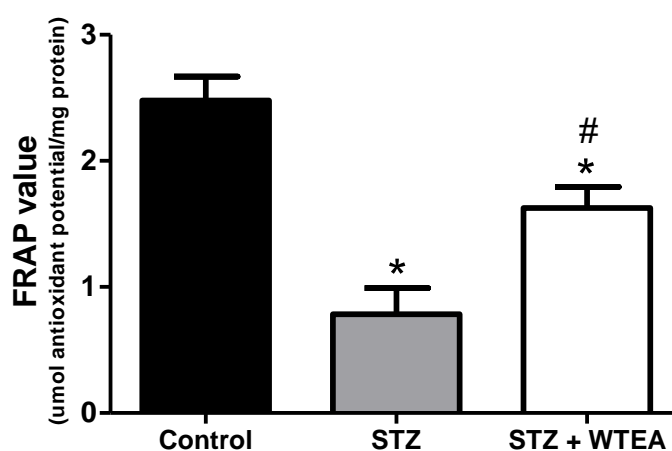


Figure 4.3.1. Effect of white tea consumption by STZ-induced diabetic rats in the antioxidant power of the heart tissue. FRAP value in ( $\mu\text{mol}$ ) of antioxidant potential/mg protein of the control in the STZ-induced diabetic rats and the STZ-induced diabetic rats consuming WTEA (STZ+WTEA), measured in the heart tissue. Results are presented as mean  $\pm$  SEM (n=6 for each condition). Significant results ( $p < 0.05$ ) are as indicated: \* vs. control; # vs. STZ group.

#### 4.4. White tea consumption decreases lipid peroxidation in hearts of STZ-induced diabetic rats

Using the TBARS assay, that measures the quantity of malondialdehyde (MDA), it was possible to determine the lipid peroxidation in hearts from rats of the control group and also in STZ-induced diabetic rats with and without WTEA consumption. Our results provide evidence that there is a decrease in the lipid peroxidation promoted by WTEA consumption in STZ-induced diabetic rats. The TBARS levels were 0.013 nmol/mg of protein in rats from the control group and were significantly increased to 0.021 nmol/mg of protein in STZ induced diabetic rats which correspond to a 1.6 fold increase. Interestingly, WTEA consumption by STZ-induced diabetic rats restored the TBARS levels to 0.015 nmol/mg of protein, a similar value to the one detected in hearts from rats of the control group (Figure 4.4.1.).

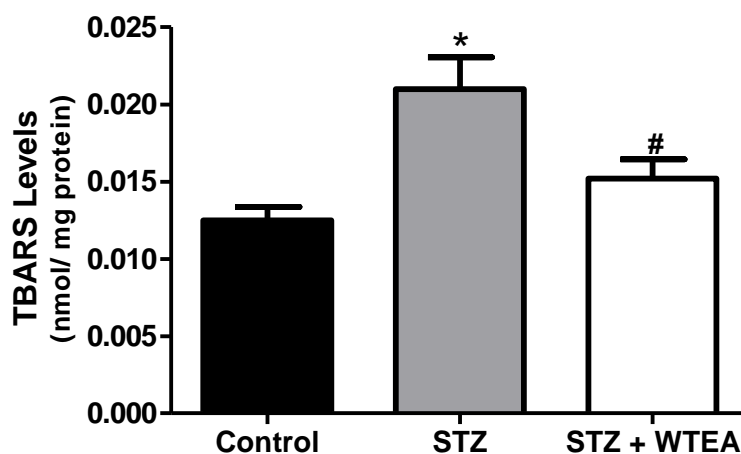


Figure 4.4.1. Effect of white tea consumption on the heart tissue lipid peroxidation in STZ-induced diabetic rats. Figure shows thiobarbituric acid reactive substance (TBARS) in control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats consuming WTEA (STZ+WTEA). Results are expressed in nmol/mg tissue and are presented as mean  $\pm$  SEM (n=6 for each condition). Significant results ( $p < 0.05$ ) are as indicated: \* vs. control; # vs. STZ group.

#### 4.5. White tea consumption slightly decreases carbonyl group formation in hearts from STZ-induced diabetic rats.

The carbonyl group assay was first described by (Reznick & Packer, 1994). ROS have been implicated as an important cause of proteins modification which may lead to their rapid degradation. One marker of protein degradation by ROS is the formation of carbonyl-groups. The hearts from the STZ-induced diabetic rats presented a 2-fold increase in the carbonyl group formation relative to hearts from rats of the control group (Figure 4.5.1.). Although the WTEA consumption did not significantly decreased the carbonyl group content in hearts from STZ-induced diabetic rats, it was noted a 13% reduction when compared with STZ-induced diabetic rats that did not consumed WTEA (Figure 4.5.1.).

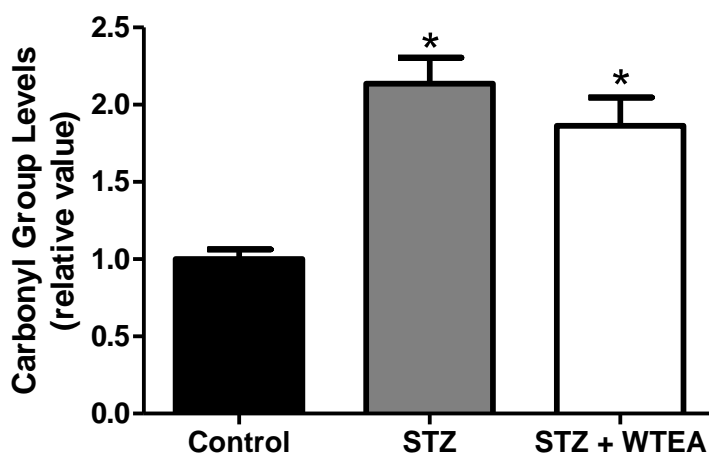


Figure 4.5.1. Effect of white tea consumption on heart tissue protein oxidation in STZ-induced diabetic rats. Figure shows carbonyl group levels in control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats consuming WTEA (STZ+WTEA). Results are expressed in relation to control and presented as mean  $\pm$  SEM (n=6 for each condition). Significant results ( $p < 0.05$ ) are as indicated: \* vs. control; # vs. STZ group.

#### **4.6. White tea consumption did not altered the glucose content in hearts of STZ-induced diabetic rats but increased lactate content**

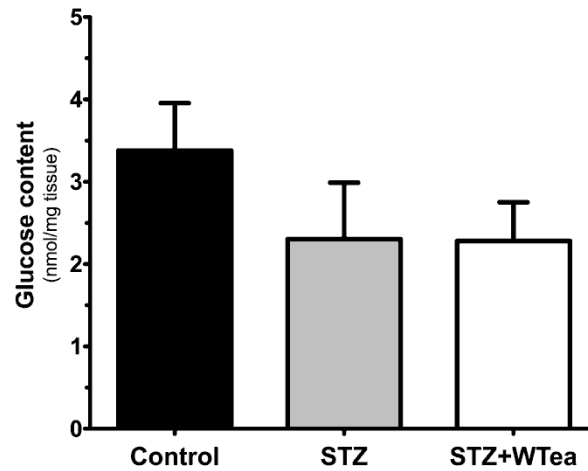
Glucose is one of the most reliable substrate for the heart. As discussed above, inside of cardiomyocytes the concentration of free glucose is lower than in blood and there is a need for a glucose gradient that favors the glucose entry. However, the glucose content was 33% lower in the STZ-induced diabetic rats in comparison to the control group (Figure 4.6.1. A). The glucose contents between the STZ-induced diabetic rats and the STZ-induced diabetic rats consuming WTEA were similar (Figure 4.6.1. A).

The lactate content in hearts from STZ-induced diabetic rats was 33% lower in comparison to the control group (Figure 4.6.1. B). The STZ-induced diabetic rats consuming WTEA presented a non-significant decrease (approximately 20%) in heart lactate content than rats of the control group. Between the STZ-induced diabetic rats consuming WTEA and the STZ-induced diabetic rats there were also no significant differences (Figure 4.6.1., Panel B).

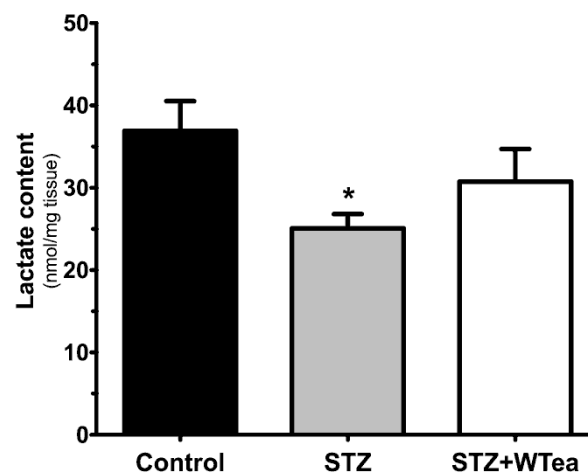
#### **4.7. White tea consumption did not alter the alanine content in hearts of STZ-induced diabetic rats but decreased the lactate/alanine ratio**

The heart alanine content in the STZ-induced diabetic rats was 15% lower than in the heart of rats from the control group (Figure 4.7.1. A). Interestingly, WTEA consumption non-significantly increased (in approximately 18%) the alanine content levels in heart of STZ-diabetic rats in comparison to the hearts of rats from the control group (Figure 4.7.1. A). The alanine content from the STZ-induced diabetic rats consuming WTEA is approximately 40% higher in relation to STZ-induced diabetic rats but this increase was also found to be statistically non-significant.

A



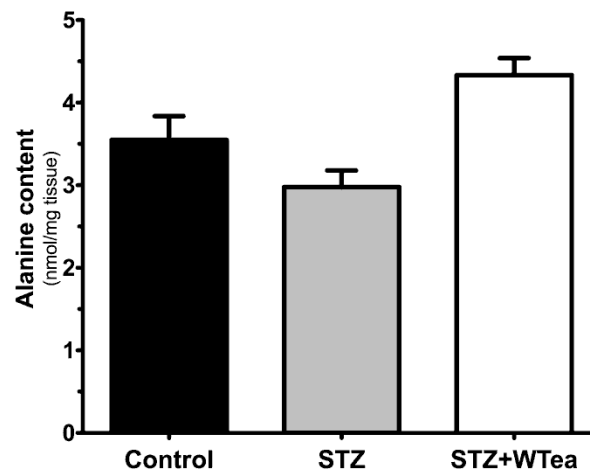
B



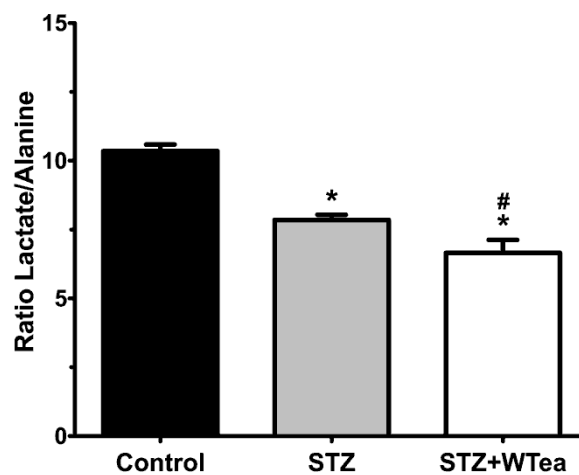
**Figure 4.6.1. Panel A:** Heart glucose content expressed in nmol/mg tissue in the control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats that consumed White tea (STZ-WTEA). **Panel B:** Lactate content in the heart tissue nmol/mg tissue of rats from control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats that consumed White tea (STZ-WTEA). Results are expressed in relation to control and presented as mean  $\pm$  SEM (n=6 for each condition). Significant results ( $p < 0.05$ ) are as indicated: \* vs. control; # vs. STZ group.

The ratio between the lactate and alanine content in the rats heart tissue was also determined (Figure 4.7.1. B). There was a significant reduction of the lactate/alanine ratio in the STZ-induced diabetic rats and in the STZ-induced diabetic rats that consumed WTEA of 25% and 31%, respectively versus the control group (Figure 4.7.1. B). There were significant differences between the STZ-induced diabetic rats and the STZ-induced diabetic rats consuming WTEA that ended up in a drop of 8% in the lactate/alanine ratio of the STZ-induced diabetic rats consuming WTEA versus the STZ-induced diabetic rats (Figure 4.7.1. B).

A



B



**Figure 4.7.1.** Panel A: Alanine content in nmol/mg tissue in heart tissue from the control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats that consumed White tea (STZ-WTEA). Panel B: Ratio between the lactate and the alanine content in the heart tissue of rats from control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats that consumed White tea (STZ-WTEA). Results are expressed in relation to control and presented as mean  $\pm$  SEM (n=6 for each condition). Significant results ( $p < 0.05$ ) are as indicated: \* vs. control; # vs. STZ group.

The acetate content from the STZ-induced diabetic rats was significant reduced in 44% in relation to the control group (Figure 4.7.3.). Besides, the STZ-induced diabetic rats consuming white tea presented a significant 25% decrease in acetate content when compared with the control group (Figure 4.7.3.). Between the STZ-induced diabetic group consuming WTEA and the STZ-induced diabetic group there were no significant differences however the acetate content of the STZ-induced diabetic group consuming white tea was 33% higher than in the STZ-induced diabetic group (Figure 4.7.3).

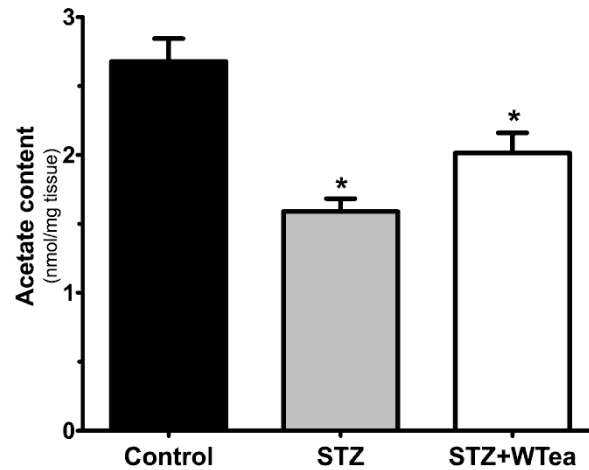


Figure 4.7.2. Acetate content in heart tissue (in nmol/mg tissue) of control, STZ-induced diabetic (STZ) and STZ-induced diabetic rats that consumed White tea (STZ-WTEA). Results are presented as mean  $\pm$  SEM (n=6 for each condition). Significant results ( $p < 0.05$ ) are as indicated: \* vs. control.

#### 4.8. White consumption increases the transcriptional levels of GLUT1, LDH and MCT4 in the STZ-induced diabetic rats

The Figure 4.8.1., illustrates the alterations in GLUT1 mRNA expression of heart tissue of each group; the control rats, STZ-induced diabetic rats and STZ-induced diabetic rats consuming WTEA.

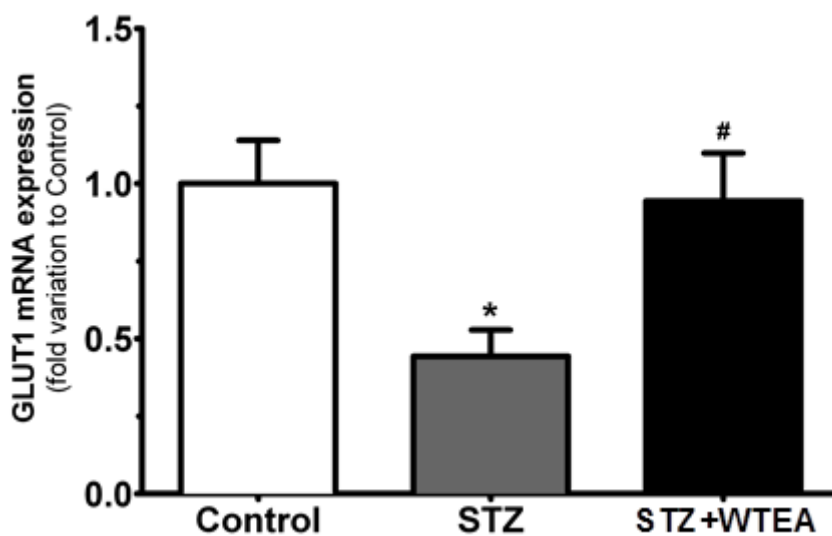


Figure 4.8.1. mRNA expression of GLUT1 in the heart tissue of Control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats consuming WTEA (STZ+WTEA). Results are presented as fold versus control (n=6 for each condition). Significant results ( $p < 0.05$ ) are as indicated: \* vs. control; # vs. STZ group.

Several reports have shown that the mRNA expression of GLUT1 are diminished in the diabetic heart (Camps et al., 1992; Young, McNulty, & Taegtmeier, 2002). As expected, in the STZ-induced diabetic rats the GLUT1 mRNA levels are significantly decreased (in 0.5-fold) comparatively to control group (Figure 4.8.1). In the STZ-induced diabetic rats consuming WTEA, the GLUT1 transcriptional levels were similar to the control group, thus being significantly higher than in STZ-induced diabetic rats.

The figure 4.8.2., illustrates the alterations in LDHA mRNA expression in heart tissue of each group; the control rats, STZ-induced diabetic rats and STZ-induced diabetic rats consuming WTEA.

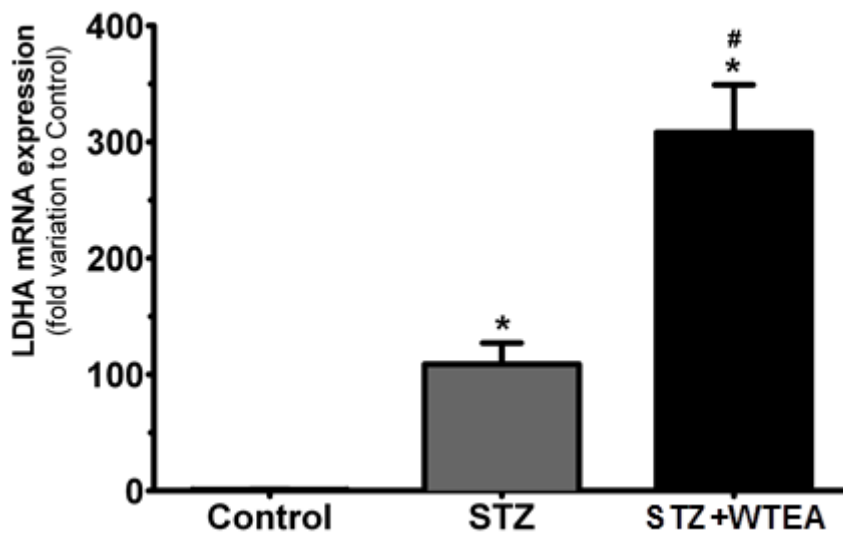


Figure 4.8.2. LDHA mRNA expression in the heart tissue of control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats consuming WTEA (STZ+WTEA). Results are presented as fold versus control (n=6 for each condition). Significant results ( $p < 0.05$ ) are as indicated: \* vs. control; # vs. STZ group.

The LDHA mRNA expression in the hearts of the STZ-induced diabetic rats is 120 fold significantly higher comparatively to the control group (Figure 4.8.2). Interestingly, WTEA consumption by the STZ-induced diabetic rats significantly increases the LDHA mRNA expression in the heart tissue about 300 times higher than the control group and about 3 times the value obtained in the STZ-induced diabetic rats that did not consume WTEA (Figure 4.8.2).

The Figure 4.8.3., illustrates the alterations in the mRNA expression of MCT4 transporters in heart tissue of each group; the control rats, STZ-induced diabetic rats and STZ-induced diabetic rats consuming WTEA.

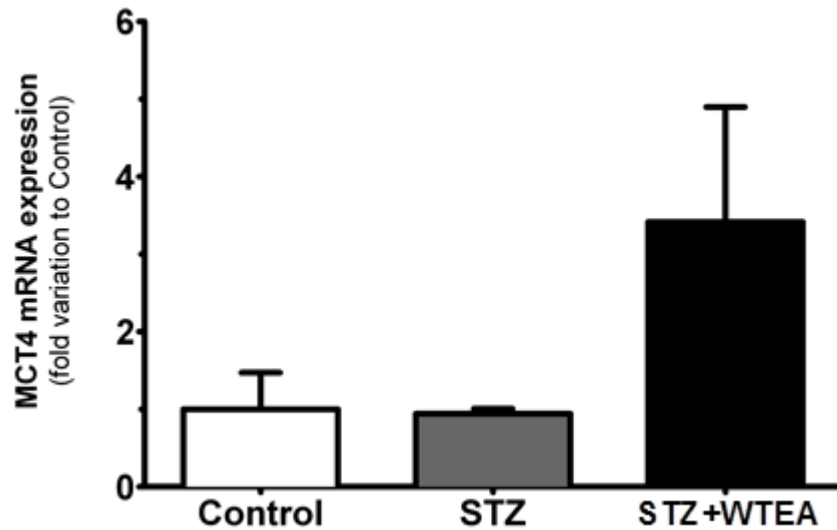


Figure 4.8.3. MCT4 mRNA expression in the heart tissue of control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats consuming WTEA (STZ+WTEA). Results are presented as fold versus control (n=6 for each condition). Significant results ( $p < 0.05$ ) are as indicated: \* vs. control, # vs. STZ group.

The MCT4 transcriptional levels in the hearts of the STZ-induced diabetic rats were similar to the value found in the control rats (Figure 4.8.3.). The transcriptional levels of MCT4 in the STZ-induced diabetic rats consuming WTEA was 2.2 times higher in comparison to the the control group and also from the STZ-induced diabetic rats (Figure 4.8.3.).

## 4.9. White tea consumption restores the LDH activity in the STZ-induced diabetic rats

The LDH activity of the heart tissue was calculated by measuring the shift on the absorbance (492 nm) of samples resulted from the conversion of a tetrazolium salt (INT) into a red formazan product as the amount of formazan formed is directly proportional to the activity of LDH on the samples. There were significant differences between the control rats and the STZ-induced diabetic rats that did not consume WTEA (Figure 4.9.1.). The LDH activity in the STZ-induced diabetic rats that did not consume WTEA was 38% lower than in control. There was an improvement of 21% from the STZ-induced diabetic rats that did not consume WTEA to the STZ-induced diabetic that did consume WTEA. There were not significant differences between the STZ-induced diabetic rats that consume WTEA to the control rats (Figure 4.9.1.).

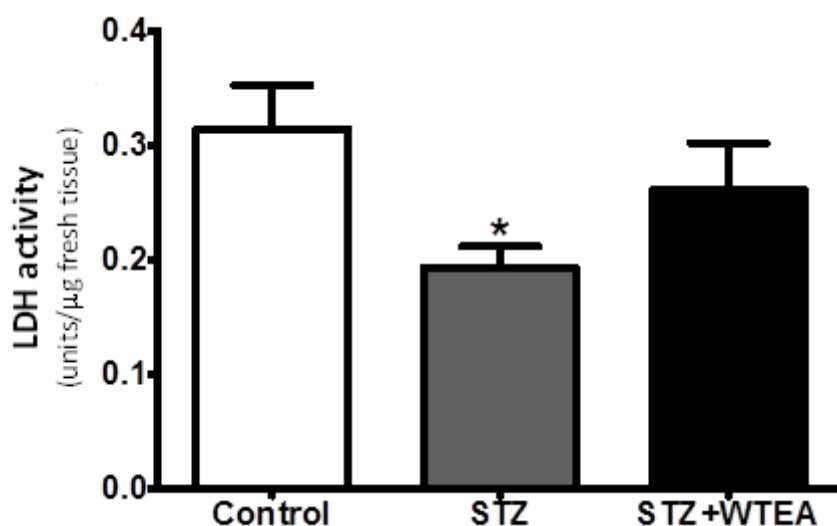


Figure 4.8.1. LDH activity in the heart tissue of the Control, STZ-induced diabetic rats (STZ) and STZ-induced diabetic rats consuming WTEA (STZ+WTEA). Results are presented as mean  $\pm$  SEM (n=6 for each condition). Significant results ( $p < 0,05$ ) are as indicated: \* vs. control; # vs. STZ group.

## **V. Discussion**

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The number of people diagnosed with Diabetes Mellitus (DM) has grown to pandemic proportions and the disease is a public health threat in modern societies. Importantly, DM is well known to have a major impact on cardiac morbidity and the WHO has reported that 80% of all diabetic deaths are due to CVD (WHO, 2004). DM is defined as a metabolic disease related with insulin deficiency or insulin resistance (Regan, Ahmed, Haider, Moschos, & Weisse, 1994) and the cardiac performance has been reported to be compromised in both, clinical and experimental DM (Cai et al., 2002; Norton, Candy, & Woodiwiss, 1996). The hyperglycemia due to insulin deficiency or insulin resistance is responsible for a wide range of cardiovascular complications such as microangiopathy, atherosclerosis, cardiomyopathy and heart failure (Dhalla, Pierce, Innes, & Beamish, 1985). Thus, it is essential to disclose the molecular mechanisms responsible for DM pathogenicity in heart, but also therapeutics that may reduce the risk for heart failure in these individuals.

Since ancient times, medicinal plants have been used for the treatment of several diseases, and even nowadays, herbal medicine is used for the prevention and control of T2D (H. Cao et al., 2007). Among the several edible and medicinal plants, plants that contain flavonoids are markedly used, for instance, in Indian and Chinese traditional medicine to treat T2D (Cheng, 2006). Tea, in general, is a great source of polyphenolic antioxidants and the polyphenolic fractions of GT are well known for their multiple pharmacological action. Epidemiologic studies have reported that the polyphenolic compounds present in tea reduce the risk of several diseases, such as cancer and cardiovascular complications (McKay & Blumberg, 2002). There are several types of tea, but WTEA is the less processed, one of the less studied and is also suggested to have the highest content of phenolic compounds. Therefore, we proposed to study the effect of WTEA consumption in the heart of diabetic rats.

We focused our study in WTEA instead of GT for two main reasons. Although there is clear evidence that tea has a cardioprotective effect in diabetic individuals, most of the studies are focused on GT and its components. Thus, there is a lack of literature concerning the biological mode of action of WTEA. Moreover, it has been suggested that WTEA has a greater potential for biological activity than GT. In fact, our preliminary results clearly show that a WTEA extract has a higher antioxidant power, as measured by the FRAP assay, than a GT extract.

The STZ-induced T2D rat model is well established since the 80s (Iwase et al., 1986). This rat model displays the metabolic characteristics of T2D in adulthood. Among those, we must note the significantly high blood glucose levels measured in the STZ-treated rats during the 60 days treatment. Furthermore, after treatment, these rats clearly developed glucose intolerance and insulin resistance, two main characteristics associated with T2D. Thus, as expected, our rats developed the etymology of T2D. It has been reported that GT consumption may improve glucose metabolism in diabetic individuals (Tsuneki et al., 2004) and some studies showed that the administration of GT to STZ-induced diabetic rats promoted blood glucose reduction (Babu et al., 2007; Fiorino et al., 2012). In our study, we could conclude that although WTEA consumption by STZ-induced T2D rats during 60 days did not significantly decrease the blood

glycaemia, glucose tolerance and insulin sensitivity were significantly improved as shown in the glucose tolerance test and insulin resistance tests. Interestingly, WTEA consumption by STZ-induced T2D rats reverted the glucose intolerance and insulin resistance to values near those of the control group. It has been reported that insulin activity can be enhanced by EGCG and other catechins, as well as theaflavins, thus improving insulin sensitivity and decreasing insulin resistance (Anderson & Polansky, 2002; Islam, 2011; T. Mackenzie, L. Leary, & W. B. Brooks, 2007). The antihyperglycemic and normoinsulinemia effect of tea has been attributed to the antioxidant properties responsible for decreased OS. Diabetic individuals are reported to present a decrease on the overall antioxidant potential (Rahimi et al., 2005), because chronic hyperglycemia causes OS, which is mostly due to increased ROS production by mitochondria (Brownlee, 2001).

Epidemiological studies consistently show that tea consumption is associated with reduced risk of CVD (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Nakachi, Matsuyama, Miyake, Suganuma, & Imai, 2000), but the defense or preventive action of tea in the heart remains largely unknown. Our results show that STZ-induced T2D individuals present a significant increase in cardiac lipid peroxidation and protein oxidation. Interestingly, WTEA consumption restored lipid peroxidation in the heart tissue to control values and protein oxidation was non-significantly decreased by WTEA consumption in hearts from diabetic individuals. These results were expected, since the favorable effects of tea consumption are correlated with the antioxidant properties of its components (Higdon & Frei, 2003). However, the protection exerted by WTEA consumption was only partial, as elevated protein oxidation levels seems to continue to occur. Others have also reported only partial protective effect of tea consumption against DM-induced oxidative damage (Juskiewicz et al., 2008). This could be due to variations in severity and duration of the disease. Further, to have a more detailed analysis of the WTEA consumption antioxidant protective effect on the heart of diabetic individuals, it is imperative to measure some antioxidant defenses such as glutathione, since the overall oxidative level is the balance between ROS production and the antioxidant defenses.

The heart functions as a pump with a continuous energetic need and thus there is a high basal level of energy that is needed to maintain the contractile activity. The oxidation of fatty acids in the heart accounts for more than 80% of ATP production under physiological conditions (Opie, Mansford, & Owen, 1971). Noteworthy, though glucose is not the main fuel for the heart, its consumption is significantly higher in this organ than in any other. Besides, in pathological conditions such as DM, glucose metabolism plays a key regulatory role in overall heart metabolic behavior, since in the heart the glucose transport and the functioning of glycolytic metabolic enzymes are sensitive to insulin fluctuations (Manchester, Kong, Nerbonne, Lowry, & Lawrence, 1994). Impaired glucose metabolism contributes to cardiac dysfunction in diabetic individuals. The rate of flux, the mRNA and protein expression as well as enzymatic functioning of key metabolic proteins/enzymes exert a tight control over cardiac metabolic performance. The glycolytic substrate is derived from exogenous glucose.

Previous reports have shown that the heart of diabetic individuals, during the development of the disease, faces membrane permeability derangements and there are ultrastructure alterations that may result in metabolites accumulation in the heart and severe injury (Dhalla et al., 1985). Our results show that glucose content is similar in the heart of control, STZ-induced T2D rats and STZ-induced T2D rats drinking WTEA. However, lactate content was significantly decreased in the hearts of STZ-induced T2D rats. The myocardium only becomes a net lactate consumer when glycolysis is not normally functioning and pyruvate oxidation is impaired as happens in poorly controlled diabetic individuals (Avogaro et al., 1990; Hall, Stanley, et al., 1996; Kaijser & Berglund, 1992; W. C. Stanley, 1991; W. C. Stanley, Lopaschuk, & McCormack, 1997). Therefore, the decrease in lactate content in STZ-induced T2D rats provides evidence that the heart is consuming lactate due to impaired glycolytic flux. The WTEA consumption increased the lactate content in heart tissue of STZ-induced T2D rats evidencing a possible mechanism by which WTEA consumption can exert its protection to cardiovascular disorders induced by diabetic conditions. Although the alanine content was not found to be altered, we must note that the lactate/alanine ratio was significantly decreased in STZ-induced T2D rats. This ratio is often used as an index of redox state of the cell (O'Donnell, Kudej, LaNoue, Vatner, & Lewandowski, 2004). The reduction of pyruvate into lactate or its conversion into alanine is related to the re-oxidation of cytosolic NADH into NAD<sup>+</sup> (L. Rato et al., 2012). Thus, the lactate/alanine ratio reflects the NADH/NAD<sup>+</sup> ratio. Our results show that STZ-induced T2D rats present a smaller ratio which could suggest that these hearts could present lower oxidative stress. However one cannot disregard that the hearts from these rats present lower lactate content which is associated with impaired glycolytic flux. Besides, caution should be taken when analyzing this ratio in the heart because it is well known that there is a compartmentalization of glycolysis and glycogenolysis (Anousis, Carvalho, Zhao, Malloy, & Sherry, 2004) making it difficult to infer about intracellular cytosolic redox by analyzing simple metabolic pathways (Carvalho et al., 2004). The WTEA consumption significantly decreased this ratio. We focused our work on oxidative stress damage and measured tissue antioxidant potential but oxidative stress is a measure of ROS production and antioxidant defenses. In fact, prior studies using cardiac tissue or cardiomyocytes from diabetic rats showed important alterations in antioxidant defenses (Cai et al., 2002; Dhalla, Liu, Panagia, & Takeda, 1998). Besides, the administration of tea polyphenols has been reported to provide cellular protection against ROS through prevention or attenuation of the decrease in antioxidant defenses induced by DM (Liao, Kao, & Hiipakka, 2001). Thus, it is expected that some antioxidant defenses, such as antioxidant enzymes, may be altered to protect the main cellular functions.

Acetate is primarily metabolized to originate Acetyl-CoA that is then used to maintain CAC. Our results show that hearts from STZ-induced T2D rats present lower content in acetate evidencing a possible stimulation of CAC. Several studies have reported that the development of cardiomyopathies in diabetic hearts is due to accelerated fatty acids oxidation and CAC stimulation (How et al., 2006; Rodrigues, Cam, & McNeill, 1998). Pharmacological inhibition

of fatty acid metabolism and increase of glucose metabolism improves the contractile function in diabetic individuals (Fragasso et al., 2003; Tahiliani, Lopaschuk, & McNeill, 1984). In the heart, the glycolytic pathway is controlled by glucose uptake that is regulated by the transmembrane glucose gradient and the content of glucose transporters. GLUT1 transporters are constitutively expressed in the heart while GLUT4 is present in intracellular vesicles and translocates to the membrane in response to insulin fluctuations (Ardehali et al., 2012). Although the ratio of GLUT1/GLUT4 is approximately 1:3 (Brownsey, Boone, & Allard, 1997), it has been consistently reported that STZ-induced DM does not change GLUT4 levels (R. Burcelin et al., 1993; Camps et al., 1992). Therefore we focused on GLUT1 levels. Our results show that STZ-induced T2D downregulates GLUT1 levels that are restored by WTEA consumption. Since glucose uptake is regulated by its gradient, STZ-induced T2D rats appear to present a protective mechanism against increased glucose uptake by downregulating GLUT1 levels.

Once glucose enters the heart, is metabolized into pyruvate that is then converted into lactate by LDH. As hyperglycemia is well known to result in increased glucose myocardial uptake (Camps et al., 1992; Hall, Henderson, Hernandez, Kellerman, & Stanley, 1996) due to increase gradient for glucose across the sarcolemmal membrane, we expected that LDH expression could be increased. Indeed, LDH expression was significantly upregulated in hearts from STZ-induced T2D rats. Noteworthy, WTEA consumption greatly increased LDH expression. This needs further investigation since it may point a crucial role for LDH in diabetic individuals and WTEA consumption is able to modulate LDH expression. Interestingly, LDH activity was only found to be decreased in STZ-induced T2D rats. Assuming that LDH activity in the heart mainly converts pyruvate to lactate, the LDH activity decrease is consistent with the decrease in lactate content in the hearts from STZ-induced T2D rats. One can conclude that production of lactate in hearts from STZ-induced T2D rats is impaired and WTEA consumption is able to restore lactate production by modulation of LDH expression and activity. More studies are needed to confirm these regulatory mechanisms since many metabolic enzymes and transporters are redundant or expressed in great excess without any specific role and therefore do not regulate the flow through the pathway (William C Stanley et al., 2005). Several authors discuss that it is possible to have dramatic changes in the levels of enzymes and transporters without a real change in substrate flux (William C Stanley et al., 2005)).

The constituents of WTEA may explain the protective effects discussed. For instance, tea polyphenols participate in Vitamin E recycling (Zhu, Huang, Tsang, & Chen, 1999) and complement the action of glutathione. Indeed, the antihyperglycemic and antioxidant action of tea consumption is usually attributed to the antioxidant potential reported and discussed in several studies (P. V. A. Babu et al., 2006a, 2006c). Tea Cs have also been consistently reported to increase the overall antioxidant activity of plasma (Yokozawa, Nakagawa, & Kitani, 2002). Therefore, the protective effect of tea may be not only due to a single

component but due to the mixture of compounds that turn tea a powerful cocktail of substances with antioxidant properties.

## **VI. Conclusions**

Diabetic individuals present greater mortality due to CVD. Abnormalities of cardiac metabolism have been consistently reported in these individuals, evidencing that metabolic remodeling is a key factor in diabetic's health. Heart glucose transport and metabolism is impaired in diabetic individuals and the rate of glycolysis is often reported as being decreased. The regulation of glycolysis is a very complex process determined by several factors, such as intracellular glucose concentration, transcriptional rates, activity of key enzymes and transporters, and the metabolic demands for the heart correct functioning. Population-based and epidemiological studies showed that GT consumption is associated with reduced mortality due to CVD (Kuriyama et al., 2006; Nantz, Rowe, Bukowski, & Percival, 2009). Therefore, it has been suggested that tea can be a novel strategy for treatment or prevention of diabetes-induced problems in the heart (H. M. Kim & Kim, 2013). Our results show that: (1) WTEA has a higher antioxidant power than GT; (2) WTEA consumption restored insulin sensitivity and glucose tolerance in STZ-induced T2D rats; (3) Hearts from STZ-induced T2D rats presented a significant decrease in its antioxidant power that was ameliorated by WTEA consumption; (4) Lipid peroxidation in hearts from STZ-induced T2D rats was significantly increased, but WTEA consumption restored it to control values; (5) Protein oxidation in hearts was significantly increased in STZ-induced T2D rats but WTEA consumption was not able to restore it to control values; (6) Hearts from STZ-induced T2D rats presented impaired glycolysis that appeared to be ameliorated by WTEA consumption; (7) Regulation of GLUT1 expression and LDH activity may be a crucial mechanism controlled by WTEA consumption in the heart of STZ-induced T2D rats.

More studies are needed to confirm the beneficial effects of WTEA consumption to diabetic hearts however tea drinking could be a standard recommendation for those who developed T2D or are at risk for developing the disease (Campbell, 2004).

## **VII . References**

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- Aadah, M., Kjaer, M., & Jorgensen, T. (2007). Associations between overall physical activity level and cardiovascular risk factors in an adult population. *Eur J Epidemiol*, 22(6), 369-378. doi: 10.1007/s10654-006-9100-3
- Abe, I., Kashiwagi, K., & Noguchi, H. (2000). Antioxidative galloyl esters as enzyme inhibitors of *p*-hydroxybenzoate hydroxylase. *FEBS letters*, 483(2), 131-134.
- Abe, Y., Umemura, S., Sugimoto, K.-i., Hirawa, N., Kato, Y., Yokoyama, N., . . . Ishii, M. (1995). Effect of green tea rich in  $\gamma$ -aminobutyric acid on blood pressure of Dahl salt-sensitive rats. *American journal of hypertension*, 8(1), 74-79.
- Alarcón, E., Campos, A., Edwards, A., Lissi, E., & López-Alarcón, C. (2008). Antioxidant capacity of herbal infusions and tea extracts: A comparison of ORAC-fluorescein and ORAC-pyrogallol red methodologies. *Food Chemistry*, 107(3), 1114-1119.
- Alberti, K. G. M. M., & Zimmet, P. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic medicine*, 15(7), 539-553.
- Almajano, M., Vila, I., & Ginés, S. (2011). Neuroprotective effects of white tea against oxidative stress-induced toxicity in striatal cells. *Neurotoxicity research*, 20(4), 372-378.
- Almajano, M. P., Carbo, R., Jiménez, J., & Gordon, M. H. (2008). Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry*, 108(1), 55-63.
- Alschuler, L. (1998). Green tea: healing tonic. *Am J Nat Med*, 5, 28-31.
- Alves, M. G., Neuhaus-Oliveira, A., Moreira, P. I., Socorro, S., & Oliveira, P. F. (2013). Exposure to 2,4-dichlorophenoxyacetic acid alters glucose metabolism in immature rat Sertoli cells. *Reprod Toxicol*, 38, 81-88. doi: 10.1016/j.reprotox.2013.03.005
- Alves, M. G., Oliveira, P. J., & Carvalho, R. A. (2011). Substrate selection in hearts subjected to ischemia/reperfusion: role of cardioplegic solutions and gender. *NMR in Biomedicine*, 24(9), 1029-1037.
- Anderson, R. A., & Polansky, M. M. (2002). Tea enhances insulin activity. *Journal of agricultural and food chemistry*, 50(24), 7182-7186.
- Anousis, N., Carvalho, R. A., Zhao, P., Malloy, C. R., & Sherry, A. D. (2004). Compartmentation of glycolysis and glycogenolysis in the perfused rat heart. *NMR Biomed*, 17(2), 51-59. doi: 10.1002/nbm.860
- Appel, L. J., Brands, M. W., Daniels, S. R., Karanja, N., Elmer, P. J., & Sacks, F. M. (2006). Dietary approaches to prevent and treat hypertension a scientific statement from the American Heart Association. *Hypertension*, 47(2), 296-308.
- Ardehali, H., Sabbah, H. N., Burke, M. A., Sarma, S., Liu, P. P., Cleland, J. G., . . . Gheorghide, M. (2012). Targeting myocardial substrate metabolism in heart failure: potential for new therapies. *Eur J Heart Fail*, 14(2), 120-129. doi: 10.1093/eurjhf/hfr173
- Atalay, M., & Laaksonen, D. E. (2002). Diabetes, oxidative stress and physical exercise. *Journal of Sports Science and Medicine*, 1(1), 1-14.
- Avogaro, A., Nosadini, R., Doria, A., Fioretto, P., Velussi, M., Vigorito, C., . . . et al. (1990). Myocardial metabolism in insulin-deficient diabetic humans without coronary artery disease. *Am J Physiol*, 258(4 Pt 1), E606-618.
- Babu, P., Sabitha, K., & Shyamaladevi, C. (2006). Green tea extract impedes dyslipidemia and development of cardiac dysfunction in streptozotocin-diabetic rats. *Clinical and Experimental Pharmacology and Physiology*, 33(12), 1184-1189.
- Babu, P. V. A., Sabitha, K. E., & Shyamaladevi, C. S. (2006a). Green tea impedes dyslipidemia, lipid peroxidation, protein glycation and ameliorates  $\text{Ca}^{2+}$ -ATPase and  $\text{Na}^+/\text{K}^+$ -ATPase activity in the heart of streptozotocin-diabetic rats. *Chemico-biological interactions*, 162(2), 157-164.
- Babu, P. V. A., Sabitha, K. E., & Shyamaladevi, C. S. (2006b). Therapeutic effect of green tea extract on advanced glycation and cross-linking of collagen in the aorta of streptozotocin diabetic rats. *Clinical and Experimental Pharmacology and Physiology*, 33(4), 351-357.

- Babu, P. V. A., Sabitha, K. E., & Shyamaladevi, C. S. (2006c). Therapeutic effect of green tea extract on oxidative stress in aorta and heart of streptozotocin diabetic rats. *Chemico-biological interactions*, 162(2), 114-120.
- Babu, P. V. A., Sabitha, K. E., Srinivasan, P., & Shyamaladevi, C. S. (2007). Green tea attenuates diabetes induced Maillard-type fluorescence and collagen cross-linking in the heart of streptozotocin diabetic rats. *Pharmacological research*, 55(5), 433-440.
- Balaban, R. S. (1990). Regulation of oxidative phosphorylation in the mammalian cell. *American Journal of Physiology-Cell Physiology*, 258(3), C377-C389.
- Balentine, D. A., Wiseman, S. A., & Bouwens, L. C. (1997). The chemistry of tea flavonoids. *Critical Reviews in Food Science & Nutrition*, 37(8), 693-704.
- Baliga, V., & Sapsford, R. (2009). Review article: Diabetes mellitus and heart failure—an overview of epidemiology and management. *Diabetes and Vascular Disease Research*, 6(3), 164-171.
- Baptista, J. A., da P Tavares, J. F., & Carvalho, R. C. (1999). Comparative study and partial characterization of Azorean green tea polyphenols. *Journal of Food Composition and Analysis*, 12(4), 273-287.
- Baptista, J. A., Tavares, J. F. d. P., & Carvalho, R. C. (1998). Comparison of catechins and aromas among different green teas using HPLC/SPME-GC. *Food Research International*, 31(10), 729-736.
- Barranco Quintana, J. L., Allam, M. F., Del Castillo, A. S., & Navajas, R. F.-C. (2009). Parkinson's disease and tea: a quantitative review. *Journal of the American College of Nutrition*, 28(1), 1-6.
- Bartlett, A. (2004). Fine teas flower in the bay area. *New York Times*, 8.
- Benzie, I. F., & Strain, J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239(1), 70-76.
- Bhattacharyya, A., Mandal, D., Lahiry, L., Sa, G., & Das, T. (2004). Black tea protects immunocytes from tumor-induced apoptosis by changing Bcl-2/Bax ratio. *Cancer letters*, 209(2), 147-154.
- Blanck, H., Dietz, W., Galuska, D., Gillespie, C., Hamre, R., Khan, L. K., . . . Mokdad, A. (2006). State-specific prevalence of obesity among adults-United States, 2005 (Vol. 296, pp. 1959-1960): AMER MEDICAL ASSOC 515 N STATE ST, CHICAGO, IL 60610-0946 USA.
- Boccarda, F., & Cohen, A. (2004). Interplay of diabetes and coronary heart disease on cardiovascular mortality. *Heart*, 90(12), 1371.
- Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition reviews*, 56(11), 317-333.
- Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature*, 414(6865), 813-820. doi: 10.1038/414813a
- Brownsey, R. W., Boone, A. N., & Allard, M. F. (1997). Actions of insulin on the mammalian heart: metabolism, pathology and biochemical mechanisms. *Cardiovasc Res*, 34(1), 3-24.
- Burcelin, R., Printz, R. L., Kande, J., Assan, R., Granner, D. K., & Girard, J. (1993). Regulation of glucose transporter and hexokinase II expression in tissues of diabetic rats. *Am J Physiol*, 265(3 Pt 1), E392-401.
- Burcelin, R., Rolland, E., Dolci, W., Germain, S., Carrel, V., & Thorens, B. (1999). Encapsulated, Genetically Engineered Cells, Secreting Glucagon-like Peptide-1 for the Treatment of Non-insulin-dependent Diabetes Mellitus. *Annals of the New York Academy of Sciences*, 875(1), 277-285.
- Cai, L., Li, W., Wang, G., Guo, L., Jiang, Y., & Kang, Y. J. (2002). Hyperglycemia-induced apoptosis in mouse myocardium: mitochondrial cytochrome C-mediated caspase-3 activation pathway. *Diabetes*, 51(6), 1938-1948.
- Campbell, A. P. (2004). Time for tea? *Diabetes Self Manag*, 21(2), 8-10, 12.

- Camps, M., Castello, A., Munoz, P., Monfar, M., Testar, X., Palacin, M., & Zorzano, A. (1992). Effect of diabetes and fasting on GLUT-4 (muscle/fat) glucose-transporter expression in insulin-sensitive tissues. Heterogeneous response in heart, red and white muscle. *Biochem. J*, *282*, 765-772.
- Cao, H., Hininger-Favier, I., Kelly, M. A., Benaraba, R., Dawson, H. D., Coves, S., . . . Anderson, R. A. (2007). Green tea polyphenol extract regulates the expression of genes involved in glucose uptake and insulin signaling in rats fed a high fructose diet. *J Agric Food Chem*, *55*(15), 6372-6378. doi: 10.1021/jf070695o
- Cao, J., Xu, Y., Chen, J., & Klaunig, J. E. (1996). Chemopreventive effects of green and black tea on pulmonary and hepatic carcinogenesis. *Toxicological Sciences*, *29*(2), 244-250.
- Carvalho, R. A., Rodrigues, T. B., Zhao, P., Jeffrey, F. M., Malloy, C. R., & Sherry, A. D. (2004). A <sup>13</sup>C isotopomer kinetic analysis of cardiac metabolism: influence of altered cytosolic redox and [Ca<sup>2+</sup>]<sub>o</sub>. *Am J Physiol Heart Circ Physiol*, *287*(2), H889-895. doi: 10.1152/ajpheart.00976.2003
- Checkoway, H., Powers, K., Smith-Weller, T., Franklin, G. M., Longstreth, W., & Swanson, P. D. (2002). Parkinson's disease risks associated with cigarette smoking, alcohol consumption, and caffeine intake. *American journal of epidemiology*, *155*(8), 732-738.
- Cheng, T. O. (2000). Tea is good for the heart. *Archives of internal medicine*, *160*(15), 2397.
- Cheng, T. O. (2006). All teas are not created equal: the Chinese green tea and cardiovascular health. *International journal of cardiology*, *108*(3), 301.
- Choi, J.-H., Chai, Y.-M., Joo, G.-J., Rhee, I.-K., Lee, I.-S., Kim, K.-R., . . . Rhee, S.-J. (2004). Effects of green tea catechin on polymorphonuclear leukocyte 5'-lipoxygenase activity, leukotriene B4 synthesis, and renal damage in diabetic rats. *Annals of Nutrition and Metabolism*, *48*(3), 151-155.
- Cleeman, J., Grundy, S., Becker, D., & Clark, L. (2001). Expert panel on Detection, Evaluation and Treatment of High blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III). *Jama*, *285*(19), 2486-2497.
- Commenges, D., Scotet, V., Renaud, S., Jacqmin-Gadda, H., Barberger-Gateau, P., & Dartigues, J.-F. (2000). Intake of flavonoids and risk of dementia. *European journal of epidemiology*, *16*(4), 357-363.
- Costa, R. M., Magalhães, A. S., Pereira, J. A., Andrade, P. B., Valentão, P., Carvalho, M., & Silva, B. M. (2009). Evaluation of free radical-scavenging and antihemolytic activities of quince ( *Cydonia oblonga* ) leaf: A comparative study with green tea ( *Camellia sinensis* ). *Food and chemical toxicology*, *47*(4), 860-865.
- Cunningham, J., Leffell, M., Mearkle, P., & Harmatz, P. (1995). Elevated plasma ceruloplasmin in insulin-dependent diabetes mellitus evidence for increased oxidative stress as a variable complication. *Metabolism, clinical and experimental*, *44*(8), 996-999.
- Curin, Y., & Andriantsitohaina, R. (2005). Polyphenols as potential therapeutical agents against cardiovascular diseases. *Pharmacological reports*, *57*, 97.
- Da Poian, A. T., & de Carvalho-Alves, P. C. (2005). *Hormônios e metabolismo: integração e correlações clínicas*: Atheneu.
- Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A., & Colombo, R. (2003). Protein carbonyl groups as biomarkers of oxidative stress. *Clinica Chimica Acta*, *329*(1), 23-38.
- de Mejia, E. G., Ramirez-Mares, M. V., & Puangpraphant, S. (2009). Bioactive components of tea: cancer, inflammation and behavior. *Brain, behavior, and immunity*, *23*(6), 721-731.
- Depre, C., Gaussin, V., Ponchaut, S., Fischer, Y., Vanoverschelde, J.-L., & Hue, L. (1998). Inhibition of myocardial glucose uptake by cGMP. *American Journal of Physiology-Heart and Circulatory Physiology*, *274*(5), H1443-H1449.
- Detection, E. P. O. (2001). EVALUATION, AND TREATMENT OF HIGH BLOOD CHOLESTEROL IN ADULTS. Executive summary of the third report of The National Cholesterol Education

- Program (NCEP) Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *Jama*, 285(19), 2486-2497.
- Devasagayam, T., Bloor, K., & Ramasarma, T. (2003). Methods for estimating lipid peroxidation: an analysis of merits and demerits. *Indian journal of biochemistry & biophysics*, 40(5), 300-308.
- Dhalla, N. S., Liu, X., Panagia, V., & Takeda, N. (1998). Subcellular remodeling and heart dysfunction in chronic diabetes. *Cardiovasc Res*, 40(2), 239-247.
- Dhalla, N. S., Pierce, G. N., Innes, I. R., & Beamish, R. E. (1985). Pathogenesis of cardiac dysfunction in diabetes mellitus. *Can J Cardiol*, 1(4), 263-281.
- Dias, T., Tomás, G., Teixeira, N., Alves, M., Oliveira, P., & Silva, B. White Tea (*Camellia Sinensis* (L.)): Antioxidant Properties and Beneficial Health Effects.
- Dufresne, C., & Farnworth, E. (2000). Tea, Kombucha, and health: a review. *Food Research International*, 33(6), 409-421.
- Dufresne, C. J., & Farnworth, E. R. (2001). A review of latest research findings on the health promotion properties of tea. *The Journal of nutritional biochemistry*, 12(7), 404-421.
- Eden, T. (1976). Tea. Tropical Agricultural Series: Longman Group Ltd, London, UK.
- Erba, D., Riso, P., Bordoni, A., Foti, P., Biagi, P. L., & Testolin, G. (2005). Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans. *The Journal of nutritional biochemistry*, 16(3), 144-149.
- Espinosa, C., López-Jiménez, J. Á., Cabrera, L., Larqué, E., Almajano, M. P., Arnao, M. B., . . . Pérez-Llamas, F. (2012). Protective effect of white tea extract against acute oxidative injury caused by adriamycin in different tissues. *Food Chemistry*.
- Expert Panel on detection, e., and treatment of high blood cholesterol in adults. (2002). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*, 106(25), 3143-3421.
- Facchini, F. S., Hua, N. W., Reaven, G. M., & Stoohs, R. A. (2000). Hyperinsulinemia: the missing link among oxidative stress and age-related diseases? *Free Radical Biology and Medicine*, 29(12), 1302-1306.
- Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C. I., Angioni, A., Dessi, S., . . . Cabras, P. (2007). Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts. *Journal of agricultural and food chemistry*, 55(3), 963-969.
- Florentino, A., D'Abrosca, B., Pacifico, S., Mastellone, C., Piscopo, V., Caputo, R., & Monaco, P. (2008). Isolation and structure elucidation of antioxidant polyphenols from quince (*Cydonia vulgaris*) peels. *Journal of agricultural and food chemistry*, 56(8), 2660-2667.
- Fiorino, P., Evangelista, F. S., Santos, F., Motter Magri, F. M., Delorenzi, J. C., Ginoza, M., & Farah, V. (2012). The effects of green tea consumption on cardiometabolic alterations induced by experimental diabetes. *Exp Diabetes Res*, 2012, 309231. doi: 10.1155/2012/309231
- Firenzuoli, F., Gori, L., Crupi, A., & Neri, D. (2004). Flavonoids: risks or therapeutic opportunities?]. *Recenti progressi in medicina*, 95(7-8), 345.
- Fischer, Y., Becker, C., & Löken, C. (1999). Purinergic inhibition of glucose transport in cardiomyocytes. *Journal of Biological Chemistry*, 274(2), 755-761.
- Fragasso, G., Piatti Md, P. M., Monti, L., Palloshi, A., Setola, E., Puccetti, P., . . . Margonato, A. (2003). Short- and long-term beneficial effects of trimetazidine in patients with diabetes and ischemic cardiomyopathy. *Am Heart J*, 146(5), E18. doi: 10.1016/s0002-8703(03)00415-0
- García-Pérez, L.-E., Álvarez, M., Dilla, T., Gil-Guillén, V., & Orozco-Beltrán, D. (2013). Adherence to Therapies in Patients with Type 2 Diabetes. *Diabetes Therapy*, 1-20.
- Giada, M., & Filho, J. (2006). The importance of dietary phenolic compounds in the promotion of human health. *Biol. Health Sci.*, 12, 7-15.

- Gould, G. W., & Holman, G. D. (1993). The glucose transporter family: structure, function and tissue-specific expression. *Biochemical Journal*, 295(Pt 2), 329.
- Gu, K., Cowie, C. C., & Harris, M. I. (1998). Mortality in adults with and without diabetes in a national cohort of the US population, 1971–1993. *Diabetes Care*, 21(7), 1138-1145.
- Gupta, R. K., Kesari, A. N., Murthy, P., Chandra, R., Tandon, V., & Watal, G. (2005). Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa* L. in experimental animals. *Journal of ethnopharmacology*, 99(1), 75-81.
- Haffner, S. M., Lehto, S., Rönnemaa, T., Pyörälä, K., & Laakso, M. (1998). Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *New England Journal of Medicine*, 339(4), 229-234.
- Hakim, I. A., Harris, R. B., Brown, S., Chow, H. S., Wiseman, S., Agarwal, S., & Talbot, W. (2003). Effect of increased tea consumption on oxidative DNA damage among smokers: a randomized controlled study. *The Journal of nutrition*, 133(10), 3303S-3309S.
- Hall, J. L., Henderson, J., Hernandez, L. A., Kellerman, L. A., & Stanley, W. C. (1996). Hyperglycemia results in an increase in myocardial interstitial glucose and glucose uptake during ischemia. *Metabolism*, 45(5), 542-549.
- Hall, J. L., Stanley, W. C., Lopaschuk, G. D., Wisneski, J. A., Pizzurro, R. D., Hamilton, C. D., & McCormack, J. G. (1996). Impaired pyruvate oxidation but normal glucose uptake in diabetic pig heart during dobutamine-induced work. *Am J Physiol*, 271(6 Pt 2), H2320-2329.
- Halliwell, B. (1997). Antioxidants and human disease: a general introduction. *Nutrition reviews*, 55(1), S44-S49.
- Hamden, K., Jaouadi, B., Carreau, S., Aouidet, A., & Elfeki, A. (2011). Therapeutic effects of soy isoflavones on alpha-amylase activity, insulin deficiency, liver-kidney function and metabolic disorders in diabetic rats. *Nat Prod Res*, 25(3), 244-255. doi: 10.1080/14786411003683117
- Hara, Y., Luo, S., Wickremasinghe, R., & Yamanishi, T. (1995a). Chemical composition of tea. *Food Rev. Int*, 11(3), 457-471.
- Hara, Y., Luo, S., Wickremasinghe, R., & Yamanishi, T. (1995b). Uses and benefits of tea. *Food Review Intl*, 11(3).
- Harbowy, M. E., Balentine, D. A., Davies, A. P., & Cai, Y. (1997). Tea chemistry. *Critical reviews in plant sciences*, 16(5), 415-480.
- Hase, T., Komine, Y., Meguro, S., Takeda, Y., Takahashi, H., Matsui, Y., . . . Shimasaki, H. (2001). Anti-obesity effects of tea catechins in humans. *Journal of Oleo Science*, 50(7), 599-605.
- Hertog, M. G., Feskens, E. J., Hollman, P. C., Katan, M. B., & Kromhout, D. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet*, 342(8878), 1007-1011.
- Higdon, J. V., & Frei, B. (2003). Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit Rev Food Sci Nutr*, 43(1), 89-143. doi: 10.1080/10408690390826464
- Hirayama, O., Takagi, M., Hukumoto, K., & Katoh, S. (1997). Evaluation of antioxidant activity by chemiluminescence. *Analytical biochemistry*, 247(2), 237-241.
- Hodgson, J. M., Burke, V., & Puddey, I. B. (2005). Acute effects of tea on fasting and postprandial vascular function and blood pressure in humans. *Journal of hypertension*, 23(1), 47-54.
- Hollman, P. C., Hertog, M. G., & Katan, M. B. (1996). Analysis and health effects of flavonoids. *Food Chemistry*, 57(1), 43-46.
- Holmes, A. G., Mesa, J. L., Neill, B. A., Chung, J., Carey, A. L., Steinberg, G. R., . . . Bruce, C. R. (2008). Prolonged interleukin-6 administration enhances glucose tolerance and

- increases skeletal muscle PPAR $\alpha$  and UCP2 expression in rats. *Journal of Endocrinology*, 198(2), 367-374.
- Hotta, Y., Huang, L., Muto, T., Yajima, M., Miyazeki, K., Ishikawa, N., . . . Ando, H. (2006). Positive inotropic effect of purified green tea catechin derivative in guinea pig hearts: The measurements of cellular Ca<sup>2+</sup> and nitric oxide release. *European journal of pharmacology*, 552(1), 123-130.
- How, O. J., Aasum, E., Severson, D. L., Chan, W. Y., Essop, M. F., & Larsen, T. S. (2006). Increased myocardial oxygen consumption reduces cardiac efficiency in diabetic mice. *Diabetes*, 55(2), 466-473.
- Hu, Z.-Q., Toda, M., Okubo, S., Hara, Y., & Shimamura, T. (1992). Mitogenic activity of (-) epigallocatechin gallate on B-cells and investigation of its structure-function relationship. *International journal of immunopharmacology*, 14(8), 1399-1407.
- Hurrell, R. F., Reddy, M., & Cook, J. D. (1999). Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *British Journal of Nutrition*, 81, 289-295.
- Iqbal, M., Sharma, S., Rezazadeh, H., Hasan, N., Abdulla, M., & Athar, M. (1996). Glutathione metabolizing enzymes and oxidative stress in ferric nitrilotriacetate mediated hepatic injury. *Redox Report*, 2(6), 385-391.
- Islam, M. (2011). Effects of the aqueous extract of white tea (*Camellia sinensis*) in a streptozotocin-induced diabetes model of rats. *Phytomedicine*, 19(1), 25-31.
- Iso, H., Date, C., Wakai, K., Fukui, M., & Tamakoshi, A. (2006). The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. *Annals of Internal Medicine*, 144(8), 554-562.
- Iwase, M., Kikuchi, M., Nuno, K., Wakisaka, M., Maki, Y., Sadoshima, S., & Fujishima, M. (1986). A new model of type 2 (non-insulin-dependent) diabetes mellitus in spontaneously hypertensive rats: diabetes induced by neonatal streptozotocin treatment. *Diabetologia*, 29(11), 808-811.
- Juskiewicz, J., Zduńczyk, Z., Jurgonski, A., Brzuzan, L., Godycka-Klos, I., & Zary-Sikorska, E. (2008). Extract of green tea leaves partially attenuates streptozotocin-induced changes in antioxidant status and gastrointestinal functioning in rats. *Nutr Res*, 28(5), 343-349. doi: 10.1016/j.nutres.2008.03.004
- Kaijser, L., & Berglund, B. (1992). Myocardial lactate extraction and release at rest and during heavy exercise in healthy men. *Acta Physiol Scand*, 144(1), 39-45. doi: 10.1111/j.1748-1716.1992.tb09265.x
- Karakaya, S., & Kavas, A. (1999). Antimutagenic activities of some foods. *Journal of the Science of Food and Agriculture*, 79(2), 237-242.
- Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*, 94(4), 550-557.
- Kaushik, G., Satya, S., Khandelwal, R. K., & Naik, S. (2010). Commonly consumed Indian plant food materials in the management of diabetes mellitus. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 4(1), 21-40.
- Kesavulu, M., Giri, R., Rao, K., & Apparao, C. (2000). Lipid peroxidation and antioxidant enzyme levels in type 2 diabetics with microvascular complications. *Diabetes & metabolism*, 26(5), 387.
- Khan, N., & Mukhtar, H. (2007). Tea polyphenols for health promotion. *Life sciences*, 81(7), 519-533.
- Kim, H. M., & Kim, J. (2013). The effects of green tea on obesity and type 2 diabetes. *Diabetes Metab J*, 37(3), 173-175. doi: 10.4093/dmj.2013.37.3.173
- Kim, W., Jeong, M. H., Cho, S. H., Yun, J. H., Chae, H. J., Ahn, Y. K., . . . Murohara, T. (2006). Effect of green tea consumption on endothelial function and circulating endothelial progenitor cells in chronic smokers. *Circulation journal: official journal of the Japanese Circulation Society*, 70(8), 1052-1057.

- Kono, S., Shinchi, K., Ikeda, N., Yanai, F., & Imanishi, K. (1992). Green tea consumption and serum lipid profiles: a cross-sectional study in northern Kyushu, Japan. *Preventive medicine, 21*(4), 526-531.
- Kuriyama, S., Shimazu, T., Ohmori, K., Kikuchi, N., Nakaya, N., Nishino, Y., . . . Tsuji, I. (2006). Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan. *JAMA: the journal of the American Medical Association, 296*(10), 1255-1265.
- Lee, M. S., & Sohn, C. B. (2009). Anti-diabetic activities of ethanol extracts from persimmon leaves. *Journal of the Korean Society for Applied Biological Chemistry, 52*(1), 96-97.
- Li, H. L., Huang, Y., Zhang, C. N., Liu, G., Wei, Y. S., Wang, A. B., . . . Liang, C. C. (2006). Epigallocatechin-3 gallate inhibits cardiac hypertrophy through blocking reactive oxidative species-dependent and -independent signal pathways. *Free Radic Biol Med, 40*(10), 1756-1775.
- Li, R. W., Douglas, T. D., Maiyoh, G. K., Adeli, K., & Theriault, A. G. (2006). Green tea leaf extract improves lipid and glucose homeostasis in a fructose-fed insulin-resistant hamster model. *Journal of ethnopharmacology, 104*(1), 24-31.
- Liao, S., Kao, Y. H., & Hiipakka, R. A. (2001). Green tea: biochemical and biological basis for health benefits. *Vitam Horm, 62*, 1-94.
- Lipinski, B. (2001). Pathophysiology of oxidative stress in diabetes mellitus. *Journal of Diabetes and its Complications, 15*(4), 203-210.
- Lloyd, S., Brocks, C., & Chatham, J. C. (2003). Differential modulation of glucose, lactate, and pyruvate oxidation by insulin and dichloroacetate in the rat heart. *American Journal of Physiology-Heart and Circulatory Physiology, 285*(1), H163-H172.
- López, V., & Calvo, M. I. (2011). White tea (*Camellia sinensis* Kuntze) exerts neuroprotection against hydrogen peroxide-induced toxicity in PC12 cells. *Plant Foods for Human Nutrition, 66*(1), 22-26.
- Łuczaj, W., & Skrzydlewska, E. (2005). Antioxidative properties of black tea. *Preventive medicine, 40*(6), 910-918.
- Luo, H., Tang, L., Tang, M., Billam, M., Huang, T., Yu, J., . . . Zhang, Z.-Q. (2006). Phase IIa chemoprevention trial of green tea polyphenols in high-risk individuals of liver cancer: modulation of urinary excretion of green tea polyphenols and 8-hydroxydeoxyguanosine. *Carcinogenesis, 27*(2), 262-268.
- MacKenzie, T., Leary, L., & Brooks, W. B. (2007). The effect of an extract of green and black tea on glucose control in adults with type 2 diabetes mellitus: double-blind randomized study. *Metabolism, 56*(10), 1340-1344.
- Mackenzie, T., Leary, L., & Brooks, W. B. (2007). The effect of an extract of green and black tea on glucose control in adults with type 2 diabetes mellitus: double-blind randomized study. *Metabolism, 56*(10), 1340-1344. doi: 10.1016/j.metabol.2007.05.018
- Magalhães, A. S., Silva, B. M., Pereira, J. A., Andrade, P. B., Valentão, P., & Carvalho, M. (2009). Protective effect of quince (*Cydonia oblonga* Miller) fruit against oxidative hemolysis of human erythrocytes. *Food and chemical toxicology, 47*(6), 1372-1377.
- Maiti, R., Jana, D., Das, U., & Ghosh, D. (2004). Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic rats. *Journal of ethnopharmacology, 92*(1), 85-91.
- Manchester, J., Kong, X., Nerbonne, J., Lowry, O. H., & Lawrence, J. C., Jr. (1994). Glucose transport and phosphorylation in single cardiac myocytes: rate-limiting steps in glucose metabolism. *Am J Physiol, 266*(3 Pt 1), E326-333.
- Mao, J. T., Nie, W.-X., Tsu, I.-H., Jin, Y.-S., Rao, J. Y., Lu, Q.-Y., . . . Serio, K. J. (2010). White Tea Extract Induces Apoptosis in Non-Small Cell Lung Cancer Cells: the Role of Peroxisome Proliferator-Activated Receptor- $\gamma$  and 15-Lipoxygenases. *Cancer Prevention Research, 3*(9), 1132-1140.

- Maritim, A., Sanders, R., & Watkins, r. J. (2003). Diabetes, oxidative stress, and antioxidants: a review. *Journal of biochemical and molecular toxicology*, 17(1), 24-38.
- Martineau, E., Tea, I., Loaec, G., Giraudeau, P., & Akoka, S. (2011). Strategy for choosing extraction procedures for NMR-based metabolomic analysis of mammalian cells. *Anal Bioanal Chem*, 401(7), 2133-2142. doi: 10.1007/s00216-011-5310-y
- Martinez-Picado, J., Savara, A. V., Sutton, L., & Richard, T. (1999). Replicative fitness of protease inhibitor-resistant mutants of human immunodeficiency virus type 1. *Journal of virology*, 73(5), 3744-3752.
- McKay, D. L., & Blumberg, J. B. (2002). The role of tea in human health: an update. *J Am Coll Nutr*, 21(1), 1-13.
- Memisoğullari, R., Taysi, S., Bakan, E., & Capoglu, I. (2003). Antioxidant status and lipid peroxidation in type II diabetes mellitus. *Cell biochemistry and function*, 21(3), 291-296.
- Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., & Devasagayam, T. P. A. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of clinical biochemistry and nutrition*, 40(3), 163.
- Moderno, P. M., Carvalho, M., & Silva, B. M. (2009). Recent patents on *Camellia sinensis*: source of health promoting compounds. *Recent Pat Food Nutr Agric*, 1(3), 182-192.
- Morrish, N., Wang, S.-L., Stevens, L., Fuller, J., & Keen, H. (2001). Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia*, 44(2), S14-S21.
- Mukhtar, H., Katiyar, S. K., & Agarwal, R. (1994). Green tea and skin--anticarcinogenic effects. *Journal of investigative dermatology*, 102(1), 3-7.
- Mustata, G. T., Rosca, M., Biemel, K. M., Reihl, O., Smith, M. A., Viswanathan, A., . . . Kern, T. S. (2005). Paradoxical effects of green tea (*Camellia sinensis*) and antioxidant vitamins in diabetic rats improved retinopathy and renal mitochondrial defects but deterioration of collagen matrix glycooxidation and cross-linking. *Diabetes*, 54(2), 517-526.
- Nagaya, N., Yamamoto, H., Uematsu, M., Itoh, T., Nakagawa, K., Miyazawa, T., . . . Miyatake, K. (2004). Green tea reverses endothelial dysfunction in healthy smokers. *Heart*, 90(12), 1485-1486.
- Nakachi, K., Matsuyama, S., Miyake, S., Suganuma, M., & Imai, K. (2000). Preventive effects of drinking green tea on cancer and cardiovascular disease: epidemiological evidence for multiple targeting prevention. *Biofactors*, 13(1-4), 49-54.
- Nakayama, M., Suzuki, K., Toda, M., Okubo, S., Hara, Y., & Shimamura, T. (1993). Inhibition of the infectivity of influenza virus by tea polyphenols. *Antiviral research*, 21(4), 289-299.
- Nantz, M. P., Rowe, C. A., Bukowski, J. F., & Percival, S. S. (2009). Standardized capsule of *Camellia sinensis* lowers cardiovascular risk factors in a randomized, double-blind, placebo-controlled study. *Nutrition*, 25(2), 147-154. doi: 10.1016/j.nut.2008.07.018
- Naziroğlu, M., & Butterworth, P. J. (2005). Protective effects of moderate exercise with dietary vitamin C and E on blood antioxidative defense mechanism in rats with streptozotocin-induced diabetes. *Canadian journal of applied physiology*, 30(2), 172-185.
- Negri, G. (2005). Diabetes melito: plantas e princípios ativos naturais hipoglicemiantes. *Brazilian Journal of Pharmaceutical Sciences*, 41(2), 121-142.
- Nihal, M., Ahmad, N., Mukhtar, H., & Wood, G. S. (2005). Anti-proliferative and proapoptotic effects of (-)-epigallocatechin-3-gallate on human melanoma: Possible implications for the chemoprevention of melanoma. *International Journal of Cancer*, 114(4), 513-521.
- Norton, G. R., Candy, G., & Woodiwiss, A. J. (1996). Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats. *Circulation*, 93(10), 1905-1912.
- O'Donnell, J. M., Kudej, R. K., LaNoue, K. F., Vatner, S. F., & Lewandowski, E. D. (2004). Limited transfer of cytosolic NADH into mitochondria at high cardiac workload. *Am J Physiol Heart Circ Physiol*, 286(6), H2237-2242. doi: 10.1152/ajpheart.01113.2003

- Odetti, P., Garibaldi, S., Noberasco, G., Aragno, I., Valentini, S., Traverso, N., & Marinari, U. (1999). Levels of carbonyl groups in plasma proteins of type 2 diabetes mellitus subjects. *Acta diabetologica*, *36*(4), 179-183.
- Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, *95*(2), 351-358.
- Okushio, K., Matsumoto, N., Korhi, T., & Susuki, M. (1996). Absorption of tea catechins into rat portal vein. *Biol Pharm Bull*.
- Oliveira, A. P., Pereira, J. A., Andrade, P. B., Valentão, P., Seabra, R. M., & Silva, B. M. (2007). Phenolic profile of *Cydonia oblonga* Miller leaves. *Journal of agricultural and food chemistry*, *55*(19), 7926-7930.
- Opie, L. H., Mansford, K. R., & Owen, P. (1971). Effects of increased heart work on glycolysis and adenine nucleotides in the perfused heart of normal and diabetic rats. *Biochem J*, *124*(3), 475-490.
- Ortsater, H., Grankvist, N., Wolfram, S., Kuehn, N., & Sjöholm, A. (2012). Diet supplementation with green tea extract epigallocatechin gallate prevents progression to glucose intolerance in db/db mice. *Nutrition & metabolism*, *9*(1), 1-10.
- Piskula, M. K., & Terao, J. (1998). Accumulation of (-)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. *The Journal of nutrition*, *128*(7), 1172-1178.
- Potenza, M. A., Marasciulo, F. L., Tarquinio, M., Tiravanti, E., Colantuono, G., Federici, A., . . . Montagnani, M. (2007). EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. *American Journal of Physiology-Endocrinology And Metabolism*, *292*(5), E1378-E1387.
- Pushparaj, P., Tan, C., & Tan, B. (2000). Effects of *Averrhoa bilimbi* leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. *Journal of ethnopharmacology*, *72*(1), 69-76.
- Rahimi, R., Nikfar, S., Larijani, B., & Abdollahi, M. (2005). A review on the role of antioxidants in the management of diabetes and its complications. *Biomedicine & Pharmacotherapy*, *59*(7), 365-373.
- Raskin, P., Jovanovic, L., Berger, S., Schwartz, S., Woo, V., & Ratner, R. (2000). Repaglinide/troglitazone combination therapy: improved glycemic control in type 2 diabetes. *Diabetes Care*, *23*(7), 979-983.
- Rato, L., Alves, M., Dias, T., Lopes, G., Cavaco, J., Socorro, S., & Oliveira, P. (2013). High-energy diets may induce a pre-diabetic state altering testicular glycolytic metabolic profile and male reproductive parameters. *Andrology*.
- Rato, L., Alves, M. G., Socorro, S., Carvalho, R. A., Cavaco, J. E., & Oliveira, P. F. (2012). Metabolic modulation induced by oestradiol and DHT in immature rat Sertoli cells cultured in vitro. *Biosci Rep*, *32*(1), 61-69. doi: 10.1042/bsr20110030
- Regan, T. J., Ahmed, S., Haider, B., Moschos, C., & Weisse, A. (1994). Diabetic cardiomyopathy: experimental and clinical observations. *N J Med*, *91*(11), 776-778.
- Reznick, A. Z., & Packer, L. (1994). [38] Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. *Methods in enzymology*, *233*, 357-363.
- Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, *20*(7), 933-956.
- Rietveld, A., & Wiseman, S. (2003). Antioxidant effects of tea: evidence from human clinical trials. *The Journal of nutrition*, *133*(10), 3285S-3292S.
- Robinson, C., Keshavarzian, A., Pasco, D., Frommel, T., Winship, D., & Holmes, E. (1999). Determination of protein carbonyl groups by immunoblotting. *Analytical biochemistry*, *266*(1), 48-57.
- Rodrigues, B., Cam, M. C., & McNeill, J. H. (1998). Metabolic disturbances in diabetic cardiomyopathy. *Mol Cell Biochem*, *180*(1-2), 53-57.

- Roghani, M., & Baluchnejadmojarad, T. (2010). Hypoglycemic and hypolipidemic effect and antioxidant activity of chronic epigallocatechin-gallate in streptozotocin-diabetic rats. *Pathophysiology*, *17*(1), 55-59.
- Ryu, O., Lee, J., Lee, K., Kim, H., Seo, J., Kim, S., . . . Choi, K. (2006). Effects of green tea consumption on inflammation, insulin resistance and pulse wave velocity in type 2 diabetes patients. *Diabetes research and clinical practice*, *71*(3), 356-358.
- Sacks, F. M., Svetkey, L. P., Vollmer, W. M., Appel, L. J., Bray, G. A., Harsha, D., . . . Simons-Morton, D. G. (2001). Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. *New England Journal of Medicine*, *344*(1), 3-10.
- Sadakata, S., Fukao, A., & Hisamichi, S. (1992). Mortality among female practitioners of Chanoyu (Japanese" tea-ceremony"). *The Tohoku journal of experimental medicine*, *166*(4), 475.
- Santana-Rios, G., Orner, G. A., Amantana, A., Provost, C., Wu, S.-Y., & Dashwood, R. H. (2001). Potent antimutagenic activity of white tea in comparison with green tea in the *Salmonella* assay. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *495*(1), 61-74.
- Sasazuki, S., Kodama, H., Yoshimasu, K., Liu, Y., Washio, M., Tanaka, K., . . . Doi, Y. (2000). Relation between green tea consumption and the severity of coronary atherosclerosis among Japanese men and women. *Annals of epidemiology*, *10*(6), 401-408.
- Seeram, N. P., Henning, S. M., Niu, Y., Lee, R., Scheuller, H. S., & Heber, D. (2006). Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity. *Journal of agricultural and food chemistry*, *54*(5), 1599-1603.
- Shaw, J., Sicree, R., & Zimmet, P. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes research and clinical practice*, *87*(1), 4-14.
- Silva, B., Valentão, P., Seabra, R., & Andrade, P. (2008). Quince (*Cydonia oblonga* Miller): an interesting dietary source of bioactive compounds. *Food Chemistry Research Developments*, 243-266.
- Sochor, J., Ruttkay-Nedecky, B., Babula, P., Adam, V., Hubalek, J., & Kizek, R. (2012). Automation of Methods for Determination of Lipid Peroxidation. *lipids*, *34*, 45.
- Sohal, R. S., & Weindruch, R. (1996). Oxidative stress, caloric restriction, and aging. *Science*, *273*(5271), 59-63.
- Sohn, E., Kim, J., Kim, C.-S., Kim, Y. S., Jang, D. S., & Kim, J. S. (2010). Extract of the aerial parts of *Aster koraiensis* reduced development of diabetic nephropathy via anti-apoptosis of podocytes in streptozotocin-induced diabetic rats. *Biochemical and biophysical research communications*, *391*(1), 733-738.
- Song, E.-K., Hur, H., & Han, M.-K. (2003). Epigallocatechin gallate prevents autoimmune diabetes induced by multiple low doses of streptozotocin in mice. *Archives of pharmacological research*, *26*(7), 559-563.
- Sowers, J. R. (1998). Diabetes mellitus and cardiovascular disease in women. *Archives of internal medicine*, *158*(6), 617.
- Sowers, J. R., Epstein, M., & Frohlich, E. D. (2001). Diabetes, hypertension, and cardiovascular disease an update. *Hypertension*, *37*(4), 1053-1059.
- Stadler, K., Jenei, V., von Bölcsházy, G., Somogyi, A., & Jakus, J. (2003). Increased nitric oxide levels as an early sign of premature aging in diabetes. *Free Radical Biology and Medicine*, *35*(10), 1240-1251.
- Stanley, W. C. (1991). Myocardial lactate metabolism during exercise. *Med Sci Sports Exerc*, *23*(8), 920-924.
- Stanley, W. C., Lopaschuk, G. D., & McCormack, J. G. (1997). Regulation of energy substrate metabolism in the diabetic heart. *Cardiovasc Res*, *34*(1), 25-33.
- Stanley, W. C., Recchia, F. A., & Lopaschuk, G. D. (2005). Myocardial substrate metabolism in the normal and failing heart. *Physiological reviews*, *85*(3), 1093-1129.

- Subramanian, N., Venkatesh, P., Ganguli, S., & Sinkar, V. P. (1999). Role of polyphenol oxidase and peroxidase in the generation of black tea theaflavins. *Journal of agricultural and food chemistry*, 47(7), 2571-2578.
- Taguri, T., Tanaka, T., & Kouno, I. (2004). Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. *Biological and Pharmaceutical Bulletin*, 27(12), 1965-1969.
- Tahiliani, A. G., Lopaschuk, G. D., & McNeill, J. H. (1984). Effect of insulin treatment on long-term diabetes-induced alteration of myocardial function. *Gen Pharmacol*, 15(6), 545-547.
- Tan, L. C., Koh, W.-P., Yuan, J.-M., Wang, R., Au, W.-L., Tan, J. H., . . . Mimi, C. Y. (2008). Differential effects of black versus green tea on risk of Parkinson's disease in the Singapore Chinese Health Study. *American journal of epidemiology*, 167(5), 553-560.
- Tokunaga, S., White, I. R., Frost, C., Tanaka, K., Kono, S., Tokudome, S., . . . Zakouji, H. (2002). Green tea consumption and serum lipids and lipoproteins in a population of healthy workers in Japan. *Annals of epidemiology*, 12(3), 157-165.
- Tsuneki, H., Ishizuka, M., Terasawa, M., Wu, J.-B., Sasaoka, T., & Kimura, I. (2004). Effect of green tea on blood glucose levels and serum proteomic patterns in diabetic (db/db) mice and on glucose metabolism in healthy humans. *BMC pharmacology*, 4(1), 18.
- Underwood, A. H., & Newsholme, E. (1965). Properties of phosphofructokinase from rat liver and their relation to the control of glycolysis and gluconeogenesis. *Biochem. J*, 95, 868-875.
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*, 39(1), 44-84.
- Valko, M., Rhodes, C., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions*, 160(1), 1-40.
- Weber, J. M., Ruzindana-Umunyana, A., Imbeault, L., & Sircar, S. (2003). Inhibition of adenovirus infection and adenain by green tea catechins. *Antiviral research*, 58(2), 167-173.
- Wheeler, D. S., & Wheeler, W. J. (2004). The medicinal chemistry of tea. *Drug development research*, 61(2), 45-65.
- WHO. (2004). WHO Library Cataloguing-in-Publication Data, Diabetes Action Now: An Initiative of the World Health Organization and the International Diabetes Federation.
- Widlansky, M. E., Hamburg, N. M., Anter, E., Holbrook, M., Kahn, D. F., Elliott, J. G., . . . Vita, J. A. (2007). Acute EGCG supplementation reverses endothelial dysfunction in patients with coronary artery disease. *Journal of the American College of Nutrition*, 26(2), 95-102.
- Wild, S., Roglic, G., Green, A., Sicree, R., & King, H. (2004). Global prevalence of diabetes estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27(5), 1047-1053.
- Willett, W. C. (1994). Diet and health: what should we eat? *Science*, 264(5158), 532-537.
- Wiseman, S. A., Balentine, D. A., & Frei, B. (1997). Antioxidants in tea. *Critical Reviews in Food Science & Nutrition*, 37(8), 705-718.
- Wolfram, S. (2007). Effects of green tea and EGCG on cardiovascular and metabolic health. *Journal of the American College of Nutrition*, 26(4), 373S-388S.
- Wu, L.-Y., Juan, C.-C., Ho, L.-T., Hsu, Y.-P., & Hwang, L. S. (2004). Effect of green tea supplementation on insulin sensitivity in Sprague-Dawley rats. *Journal of agricultural and food chemistry*, 52(3), 643-648.
- Yamabe, N., Yokozawa, T., Oya, T., & Kim, M. (2006). Therapeutic potential of (-)-epigallocatechin 3-O-gallate on renal damage in diabetic nephropathy model rats. *Journal of Pharmacology and Experimental Therapeutics*, 319(1), 228-236.

- Yang, C. S., Chung, J. Y., Yang, G.-y., Chhabra, S. K., & Lee, M.-J. (2000). Tea and tea polyphenols in cancer prevention. *The Journal of nutrition*, *130*(2), 472S-478S.
- Yang, C. S., Lee, M.-J., & Chen, L. (1999). Human salivary tea catechin levels and catechin esterase activities: implication in human cancer prevention studies. *Cancer Epidemiology Biomarkers & Prevention*, *8*(1), 83-89.
- Yang, Y.-C., Lu, F.-H., Wu, J.-S., Wu, C.-H., & Chang, C.-J. (2004). The protective effect of habitual tea consumption on hypertension. *Archives of internal medicine*, *164*(14), 1534.
- Yokozawa, T., Nakagawa, T., & Kitani, K. (2002). Antioxidative activity of green tea polyphenol in cholesterol-fed rats. *J Agric Food Chem*, *50*(12), 3549-3552.
- Young, M. E., McNulty, P., & Taegtmeyer, H. (2002). Adaptation and maladaptation of the heart in Diabetes: part II Potential mechanisms. *Circulation*, *105*(15), 1861-1870.
- Zhang, P., Zhang, X., Brown, J., Vistisen, D., Sicree, R., Shaw, J., & Nichols, G. (2010). Global healthcare expenditure on diabetes for 2010 and 2030. *Diabetes research and clinical practice*, *87*(3), 293.
- Zhu, Q. Y., Huang, Y., Tsang, D., & Chen, Z. Y. (1999). Regeneration of alpha-tocopherol in human low-density lipoprotein by green tea catechin. *J Agric Food Chem*, *47*(5), 2020-2025.
- Zimmet, P. Z., Alberti, K. G., & Shaw, J. E. (2005). Mainstreaming the metabolic syndrome: a definitive definition. *Med J Aust*, *183*(4), 175-176.