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Laboratory Studies on Weight Control and Prevention of Metabolic Syndrome by Green Tea¹

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Abstract

Green tea (*Camellia sinensis*, Theaceace) is the second most popular beverage in the world and has been extensively studied for its putative disease preventive effects. Green tea is characterized by the presence of a high concentrations of polyphenolic compounds known as catechins, with (–)-epigallocatechin-3-gallate (EGCG) being the most abundant and most well-studied. Metabolic syndrome (MetS) is a complex condition that is defined by the presence of elevated waist circumference, dysglycemia, elevated blood pressure, decrease serum high density lipoprotein-associated cholesterol, and increased serum triglycerides. Studies in both *in vitro* and laboratory animal models have examined the preventive effects of green tea and EGCG against the symptoms of MetS. Overall, the results of these studies have been promising and demonstrate that green tea and EGCG have preventive effects in both genetic and dietary models of obesity, insulin resistance, hypertension, and hypercholesterolemia. Various mechanisms have been proposed based on these studies and include: modulation of dietary fat absorption and metabolism, increased glucose utilization, decreased *de novo* lipogenesis, enhanced vascular responsiveness, and antioxidative effects. In the present review, we discuss the current state of the science with regard to laboratory studies on green tea and MetS. We attempt to critically evaluate the available data and point out areas for future research. Although there is a considerable amount of data available, questions remain in terms of the primary mechanism(s) of action, the dose-response relationships involved, and the best way to translate the results to human intervention studies.

¹**Abbreviations:** ACO, acetyl CoA oxidase; ALT, alanine aminotransferase; AMPK, adenosine monophosphate activated kinase; Ang, angiotensin; aP2, Adipocyte/macrophage fatty acid-binding protein; BMI, body mass index; C/EBP- α , CCAAT enhancer-binding protein- α ; CPT, carnitine palmitoyltransferase; *db/db*, BKS.Cg-Dock7m $\pm$ /Leprdb/J; DHAP, dihydroxyacetone phosphate; EC, (–)-epicatechin; ECG, (–)-epicatechin-3-gallate; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin-3-gallate; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; ERK, extracellular responsive kinase; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; GLUT, glucose transporter; GPDH, glycerol-3-phosphate dehydrogenase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; HSL, hormone-sensitive lipase; IL, interleukin; IRS, insulin receptor substrate; K_i , inhibitory constant; LDL, low-density lipoprotein; LPL, lipoprotein lipase; MCAD, medium chain acyl coA dehydrogenase; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; *ob/ob*, B6.V-Lepob/J; OGTT, oral glucose tolerance test; ORFLD, obesity-related fatty liver disease; PI3K, phosphatidylinositol 3 kinase; PEPCK, phosphoenolpyruvate carboxykinase; PPAR, peroxisome proliferator-activated receptor; SCD-1, stearoyl-CoA desaturase-1; SHR, spontaneously hypertensive rat; SOD, SREBP-1c, sterol regulatory element-binding protein-1c superoxide dismutase; STZ, streptozotocin; T1D, type 1 diabetes; T2D, type 2 diabetes; TNF, tumor necrosis factor; UCP, uncoupling protein; ZDF, Zucker diabetic fatty

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Keywords

catechins; (-)-epigallocatechin-3-gallate; green tea; metabolic syndrome; obesity

1. Introduction

Metabolic syndrome (MetS) is a significant public health problem worldwide and approximately 34% of adults in the United States meet the criteria for metabolic syndrome according to the National Cholesterol Education Program's Adult Treatment Panel III (guidelines [1]. According to the American Association of Clinical Endocrinologists and the International Diabetes Federation metabolic syndrome is defined as a complex of symptoms that includes having elevated waist circumference (> 102 cm in men and > 88 cm in women) and two or more of the following factors: elevated serum of triglycerides, dysglycemia, elevated blood pressure, and reduced serum of high-density lipoprotein (HDL) associated cholesterol [2]. Persons exhibiting MetS also commonly have obesity-related fatty liver disease (ORFLD) and ORFLD is now widely accepted as the hepatic component of the metabolic syndrome [3-5]. MetS greatly increases risk for chronic disease such as cardiovascular disease, cancer, and others.

Consumption of a high-fat diet is a strong risk factor for the development of obesity and metabolic syndrome. Epidemiological studies have shown that obesity is generally more prevalent in societies that consume a Western-style diet, which, in addition to being deficient in several nutrients, is also high in fat (30-40% of kcal in diet) [6-8]. Dietary, pharmacological, and surgical strategies have been developed in the last decade to prevent the metabolic effects of a high-fat diet. These methods control food intake, increase energy expenditure, promote fat oxidation in the body, or inhibit fat absorption into the body. Pharmacological treatment for the metabolic syndrome often consists of separate drugs targeted at the individual symptoms of the disease [9]. Despite these advances, there are still several risks associated with pharmacological and surgical intervention of obesity and the metabolic syndrome, suggesting that dietary modification may be the safest and most cost-effective option for those who are moderately obese [10-12]. To date, nutritional intervention is still the preferred method of treatment and prevention for obesity and the metabolic syndrome [9,13].

Tea (*Camellia sinensis*, Theaceae) is the second popular beverage in the world, next to water. The three main types of tea, green, oolong, and black, differ in terms of processing and chemical composition. Green tea is prepared by either steaming or pan-frying tea leaves to inactivate oxidative enzymes. In the preparation of oolong and black tea, the leaves are crushed and allowed to undergo a polyphenol oxidase-mediated oxidation known as fermentation. Many of the putative health benefits of tea are attributed to the high polyphenol content of this beverage [14]. Although all types of tea are rich in polyphenolic compounds, the processing of tea dictates the types and quantities of polyphenols that are found in each specific beverage. Whereas black tea contains oligomeric compounds known as theaflavins and thearubigens, green tea contains mainly monomeric polyphenols known as catechins. A typical brewed green tea beverage (2.5 g green tea in 250 ml hot water) contains about 240 – 320 mg catechins including (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG). Small amounts of (+) catechin and (+) gallocatechin are also present [14]. EGCG is the most abundant catechin present in green tea and accounts for approximately 30 – 50% of the catechin content [15]. In addition to the catechin constituents in green tea, caffeine is also present at relatively high amounts [14]. Studies have also indicated that caffeine may play a

role in the preventive effects of green tea against cancer, MetS and other disease states [16,17].

Green tea has been reported to have preventive effects against a number of chronic diseases including heart disease, neurodegenerative disease, cancer, and others [18,19]. The potential preventive effects of green tea and EGCG on symptoms of MetS have also been investigated in laboratory, epidemiological and intervention studies [20,21]. In this review, we summarize the laboratory-derived data on the potential effects of green tea as an agent for prevention of obesity and the metabolic syndrome with the aim of critically evaluating the results of these studies and synthesizing phenomenological and mechanistic data. The focus of this review will be on studies conducted in animal models related to MetS and we will also discuss relevant *in vitro* mechanistic data. For organizational purposes, we have subdivided the review into sections on the individual symptoms of MetS (*i.e.* obesity, diabetes/insulin resistance, hypertension, hypercholesterolemia, and ORFLD), although given the nature of the models used and the multiple symptoms involved, clear demarcations of sections are somewhat difficult to make.

2. Prevention of obesity by green tea polyphenols

Obesity is associated with increased health-care costs, reduced quality of life, and increase risk for premature death, defined as a body mass index (BMI) of 30 or greater [20]. The Centers of Disease Control and Prevention reported that during the past 20 years there has been dramatic increase in the rate of obesity in the United States and currently more than 60% of US population is overweight or obese [22]. The effects of green tea and green tea polyphenols have been examined in a number of animal models of obesity (Table 1).

Hasegawa et al. reported that oral administration of 130 mg powdered green tea daily to male Zucker rats fed a 50% sucrose diet containing 15% butter resulted in reduction of body weight gain within 2 days. In addition, rats treated with powdered green tea had significantly lowered adipose tissue weight (5 – 9% decrease) and liver weight (11% decrease) [23]. Green tea treatment (2% in the diet) reduced body fat accumulation in Sprague-Dawley rats after 14 days but did not alter body weight gain [24].

Park et al. [25] have studied the effects of green tea extract on obesity and hepatic steatosis in leptin-deficient B6.V-Lepob/J (*ob/ob*) mice. Treatment with 1% green tea extract containing 30% (w/w) total catechins for 6 weeks resulted in decreased body weight gain compared to control mice. Green tea treatment also reduced adipose tissue mass (21% decrease), hepatic lipids (13% decrease), and serum alanine aminotransferase (ALT, 25% decrease) compared to control mice. Immunohistochemical analysis showed that 1% green tea extract treatment decreased hepatic expression of the inflammatory marker, tumor necrosis factor (TNF)- α , in the liver and adipose tissue.

Studies with green tea extract are potentially confounded by the presence of caffeine. In order to determine the relative contribution of the tea polyphenols and caffeine, some investigators have examined the effects of decaffeinated green tea or compared the effects of green tea extract, tea catechins, and caffeine. A recent study compared the effects of supplementation with decaffeinated green tea powder, tea catechins, and heat-treated tea catechins in Sprague-Dawley rats [26]. All three treatments caused significantly reduced final body weight and epididymal, mesenteric and perirenal and retroperitoneal adipose tissue. Richard et al. [27] likewise studied the effects of decaffeinated green tea in both diet-induced obesity in C57BL/6J mice and genetic obesity in the *ob/ob* mouse. The results showed that administration of 2% decaffeinated green tea for 6 weeks to *ob/ob* mice significantly slowed the rate of body weight gain compared to control group, but there was no difference in C57BL/6J mice treated and control group. By contrast, caffeine was shown

to play an important role in the effects of green tea in SAMP10 mice, which develop brain atrophy and cognitive dysfunction due to accelerated senescence and are susceptible to high fat diet induced obesity. Treatment with 0.3% green tea catechins and 0.05% caffeine for 12 months improved brain atrophy, brain dysfunction and obesity whereas treatment with catechins alone did not show significant lower body weight and adipose tissue weight [28].

Many studies have focused on the effects of purified tea catechin preparations or pure EGCG. Supplementation of high-fat fed C57BL/6J mice fed with 0.2 and 0.5% tea catechin was shown to reduce body weight gain, visceral adipose tissue weight (44 – 87% decrease) and liver triglyceride (53 – 75% decrease) [29,30]. Tea catechin treatment also reduced plasma total cholesterol and plasma glucose in non-fasting condition [30]. A study in obese male Sprague-Dawley rats fed high-fat diet found that administration of 0.5% tea catechins for 8 weeks did not significantly lower the body weight or visceral adipose tissue, but did reduce by interscapular brown adipose tissue 15% [31]. Treatment of high-fat fed C57BL/6J mice with 0.32% dietary EGCG for 16 weeks reduced body weight gain by 33 – 41% compared to high fat-fed controls [32]. In addition, the EGCG-treated mice had significantly lower total visceral adipose tissue weight (37% decrease). The same group also found that EGCG treatment for 4 weeks significantly lowered the weight of the mesenteric adipose depot (36% decrease) and tended to decrease body weight (not statistically significant). Sprague-Dawley rats treated with 1% w/w TEAVIGO (90% EGCG) for 1 month showed reduction of subcutaneous adipose tissue (10% decrease), epididymal adipose tissue (5% decrease), and fed state triglyceride (11% decrease) [33]. By contrast Raederstorff et al. [34] showed that supplementation with 0.25%, 0.5% and 1.0% EGCG had no significant effect on body weight and liver weight in Wistar rats fed a high-fat, high cholesterol diet for 4 weeks, EGCG (1%) did however reduce total plasma cholesterol and non-HDL cholesterol and hepatic total cholesterol concentration by 37%, 55% and 17% respectively compared to control group.

A number of mechanisms have been proposed to explain the anti-obesity effects of green tea. One reported by several groups is related to modulation of dietary lipid absorption by green tea treatment. Yang et al. and Muramastu et al. [35,36] have reported that green tea extract and green tea catechins increase fecal lipid content in high fat-fed rats. Similar findings have been observed in high fat-fed mice. For example, EGCG supplementation (0.32% in the diet) for 16 weeks increased fecal lipid content in high fat-fed mice by 144% compared to high fat controls [32]. Treatment with 0.5% and 1.0% EGCG has also been shown to increase fecal cholesterol excretion and fecal fat excretion in high fat, high cholesterol-fed rats compared to control group [4]. Fecal lipid content was also shown to increase following treatment of *ob/ob* mice with decaffeinated green tea for six weeks, although the effect was not statistically significant. This may be due to the relatively short intervention time compared to other studies [27].

Green tea extract and tea catechins have been reported to inhibit pancreatic lipase. An *in vitro* under gastric and duodenal conditions showed that fat digestion was significantly inhibited by inclusion of 60 mg EGCG/g triolein substrate, and this effect was related to the changes in lipid emulsification in gastric or duodenal media [37]. A recent study showed that EGCG at the achievable by typical daily intake changed the physiochemical properties of a lipid emulsion by increasing its particle size and reducing the surface area [38]; such changes decreased the ability of pancreatic lipase to digest dietary fats [39]. Ikeda et al. [40] demonstrated that a mixture of catechins high in EGCG and ECG inhibited pancreatic lipase *in vitro* and suppressed postprandial serum triglyceride *in vivo* in a dose-dependent manner. It has been proposed that the hydroxyl moieties of EGCG interact with the hydrophilic head group of phosphatidylcholine at the exterior of a lipid emulsion by forming hydrogen bonds.

These interactions may lead to formation of cross-links followed by coalescence of the emulsion droplets [38].

Modulation of lipid metabolism in liver and/or adipose tissue has also been suggested as a potential mechanism by which prevents obesity. Studies have focused on the effect of green tea or EGCG on expression and activity of enzymes related to fatty acid synthesis or fatty acid oxidation. Supplementation of 1% tea catechin and heat-treated tea catechin in Sprague-Dawley rats for 23 days reduced activities of fatty acid synthase (FAS) and malic enzyme in the liver significantly where there was a tendency of reduction of glucose-6-phosphate dehydrogenase (G6PDH), carntine palmitoyltransferase (CPT) and acyl coA oxidase (ACO) activities [26]. Klaus et al. [41] also reported that dietary supplementation of 0.5% and 1% EGCG reduced leptin expression of epididymal adipose tissue and reduced expression of SCD-1, malic enzyme, and glucokinase in the liver. EGCG supplementation was shown to significantly decrease FAS, glycerol-3-phosphate acyltransferase, sterol-coenzyme A desaturase (SCD-1) mRNA expressions in adipose tissue compared to high fat-fed mice [33,41]. These enzymes are related to fatty acid synthesis such as SCD-1 is the rate-limiting enzyme in the synthesis of monounsaturated fatty acids. Reduction of fatty acid synthesis is expected to lead to reduced triglyceride deposition in the liver and adipose tissue.

Inhibition of FAS and G6PDH by green tea and EGCG have also been observed *in vitro*. Yeh et al. [42] showed that the expression of fatty acid synthase mRNA in malignant human breast carcinoma MCF-7 cells was suppressed by the addition of the green tea (60 - 120 µg/mL) and EGCG (76% decrease at 30 µM). EGCG suppressed FAS induction by epidermal growth factor (EGF) by inhibiting the phosphatidylinositol 3-kinase (PI3K)/Akt – mediated signaling. Kao et al. [43] found that EGCG inhibited glycerol-3-phosphate dehydrogenase (GPDH) with IC₅₀ value of 20 µM in a cell-free system. Also they revealed that EGCG was a noncompetitor of GPDH substrates, NADH and dihydroxyacetone phosphate (DHAP) with respective inhibition constants (K_i) of 18 and 31 µM. In both cases, the concentrations of EGCG are quite high.

Many studies have examined the effects of tea catechin or EGCG on β-oxidation. The supplementation with 0.5% tea catechin in C57BL/6J mice significantly increased β-oxidation activity in the liver, and the increases in mRNA levels of ACO and medium chain acyl-CoA dehydrogenase (MCAD) in the liver were observed [29,30]. Interestingly, no change in FAS expression was observed. Other recent studies [25,44] have shown that EGCG can modify gene expression in epididymal white adipose tissue of treated mice. The mRNA levels of adipogenic genes related to adipocyte differentiation such as peroxisome proliferator-activated receptor (PPAR)-γ, CCAAT enhancer-binding protein-α (C/EBP-α), sterol regulatory element-binding protein-1c (SREBP-1c), adipocyte fatty acid-binding protein (aP2), lipoprotein lipase (LPL), fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD-1) were significantly decreased. The similar results of gene expression related to adipocyte differentiation (SREBP-1c, FAS, and SCD-1) were found in the liver [25]. Moreover, the increase of mRNA levels of genes related to lipolysis, β-oxidation and thermogenesis which are CPT-1, uncoupling protein (UCP)2, hormone sensitive lipase (HSL) and adipose triglyceride lipase, were observed [44]. The study of Nomura et al. [31] have shown that tea catechin intake in the context of normal fat diet (12% calories from fat) significantly increased the UCP1 mRNA expression in brown adipose tissue. Similar effects have also been observed in the content of a high fat diet. UCP1 plays a role in thermogenesis in brown adipose tissue and may help explain the reduction of fat weight following treatment with tea catechins.

A recent study by Murase et al. [45] studied the effects of EGCG in BALB/c mice and the results showed that oral administration of EGCG (200 mg/kg BW) induced an increase in

AMPK α activity in the liver. Respiratory quotient values tended to decrease as determined by indirect calorimetry and it showed significant increase in fat oxidation which suggested that EGCG increased fatty acid oxidation although no change in body weight was observed. *In vitro* study, the same group reported that EGCG not only induced the increase of AMPK α but that a galloocatechin moiety or a galloyl residue, acted as AMPK activators.

3. Prevention of insulin resistance and diabetes by green tea polyphenols

Insulin resistance is an early marker of type 2 diabetes (T2D) and development of insulin resistance is associated with obesity [46]. It has been suggested that a major contributor to the development of insulin resistance is an overabundance of free fatty acids in the plasma [47]. Insulin resistance results in the interruption of insulin signaling in responsive tissues, which leads to hyperinsulinemia and ultimately T2D. Over the long term, the body is unable to produce enough insulin to overcome insulin resistance and the pancreas may reduce or stop insulin production [48]. In addition insulin resistance can result in decreased levels of lipoprotein lipase in peripheral tissues (i.e. adipose tissue and muscle). By contrast type I diabetes (T1D) begins early in life and occurs as a result of autoimmune destruction of pancreatic β -cells resulting in insulin deficiency [49]. It is predicted that the number of people with both diagnosed and undiagnosed diabetes will increase from 23.7 million in 2009 to 44.1 million in 2034 [50]. A number of studies have been conducted to examine the effects of green tea in animal models of both T1D and T2D (Table 2.).

Islam and Choi [51] showed that green tea improved glucose tolerance in streptozotocin (STZ)-treated rats. One week after STZ injection, diabetic rats (non-fasting blood glucose $\geq$ 300 mg/dL) were given low (0.5%) and high (2.0%) doses of green tea extract as the sole source of drinking fluid for 4 weeks. Green tea treatment significantly decreased blood glucose following glucose challenge in diabetic rats treated with low dose of green tea extract. Interestingly, the high dose green tea extract group actually had elevated blood glucose. The reason for this lack of dose-response was not discussed, but could indicate some level of toxicity at this higher dose.

Roghani *et al.*, examined the beneficial effects of EGCG on chemically induced T1D. Male Wistar albino rats were injected with STZ (60 mg/kg BW), after one week the rats with serum glucose higher than 250 mg/dL were treated with EGCG (25 mg/kg, i.g. daily) for 8 weeks [52]. EGCG treatment decreased serum glucose levels compared to diabetic controls and baseline values. In addition, the EGCG treatment caused a 30.9% decrease in malondialdehyde levels and a 21.3% increase in superoxide dismutase (SOD) in aortic rings compared to the diabetic control demonstrating the potential antioxidant effects of EGCG in this model.

Another study has reported that EC can also reduce the toxicity of STZ in rat pancreatic islets [53]. Rats were randomly divided into four groups: control, EC (30 mg/kg)-treated, STZ (60 mg/kg)-treated, and EC (30 mg/kg) plus STZ (60 mg/kg)-treated. Rats given EC twice a day for 6 days followed by a single injection of STZ showed no hyperglycemic effects. *In vitro* EC (0.8mM) treatment protected islet cells from STZ and potentiated compared to STZ (5mM)-treated islet cells. Although these *in vitro* results are interesting, the dose of EC used in the study is much higher than what is physiologically-achievable by tea consumption.

In addition to its effects on hyperglycemia, green tea has also been examined for its effects on diabetes-related co-pathologies including cataracts. Due to hyperglycemia and increased oxidative stress induced by diabetes, nonenzymatic glycation of proteins can occur and subsequent advanced glycation end-product can form leading to structural and functional changes of proteins and sorbitol accumulation. Male Sprague-Dawley rats injected with STZ

and treated with green tea extract (1.25%) for 3 months had reduced formation of diabetic cataracts and significantly decreased glucose, glycated lysine and sorbitol levels in the lens [54]. Levels of oxidative stress markers were also reduced.

Although the effects of green tea on T1D are interesting, the recent increases in the incidence of obesity make understanding the effects of green tea against T2D very important. Green tea and tea polyphenols have been studied using both diet-induced and genetic models. Ramadan et al. [55] examined the effect of green tea aqueous extract on male Wistar albino rats fed a cholesterol-rich diet. Treatment with 50 mg/kg - 100 mg/kg BW for 4 weeks lowered body weight (28% - 49%), serum glucose (20% - 31%), total lipid (26% - 31%), triacylglycerides (22% - 40%), phospholipid levels (32% - 38%), ALT (49% - 56%), aspartate aminotransferase (58% - 62%) and alkaline phosphatase activities (16% - 22%).

Wolfram et al. examined the effects of EGCG in two rodent genetic models of T2D [56]. Zucker Diabetic Fatty (ZDF) rats are an inbred rat model with ineffective leptin receptors and prone to T2D, glucose intolerance and obesity. Similarly, BKS.Cg-*m* *+/+* *Lepr^{db}/J* (*db/db*) mice have a leptin receptor mutation, are obese and have elevated insulin and blood glucose levels. *Db/db* mice treated with 0.25–1% dietary EGCG for 7 weeks, had dose-dependently improved oral glucose tolerance and decreased fasting glucose. EGCG-treated ZDF rats showed similar decreases in fasting blood glucose. In addition, mechanistic experiments showed that EGCG dose dependently increased glucokinase expression and decreased the expression of phosphoenolpyruvate carboxykinase (PEPCK) in the liver of *db/db* mice. The former enzyme enhances glycolysis and glucose uptake in the liver, whereas the latter enzyme functions in gluconeogenesis. EGCG treatment also increased expression of acyl-CoA oxidase-1 (ACO-1) and carnitine palmitoyl transferase-1 (CPT-1) in liver and adipose tissue of the *db/db* mice. Both enzymes are involved in fatty acid catabolism. These alterations in glucose and lipid metabolism may explain the observed improvement in glucose homeostasis.

Serisier et al. [57] showed that green tea can affect insulin sensitivity in dogs. Obese and insulin-resistant beagle dogs were treated with oral green tea extract (80 mg/kg BW per day) just before the daily meal for 12 weeks. Insulin sensitivity index was markedly increased by green tea supplementation (60% increase). Moreover, the homeostasis model for insulin resistance (HOMA-IR) was decreased by 20% after green tea treatment. Gene expression analysis in visceral and subcutaneous adipose tissue showed that expression of PPAR γ , LPL, adiponectin, and GLUT4 mRNA were dramatically elevated after 12 weeks of green tea supplementation (3-fold, 10-fold, 6-fold and 3-fold, respectively). In skeletal muscle, green tea supplementation also markedly increased the expression of PPAR α and LPL mRNA (2-fold and 3-fold) but did not increase GLUT4 mRNA. Green tea did increase however GLUT4 translocation to the plasma membrane in muscle cells.

Studies in non-diabetic animals have also supported the potential beneficial effects of green tea against diabetes. Wu et al. [58] studied the effect of green tea supplementation on insulin sensitivity in Sprague-Dawley rats given 0.5% green tea as the sole source of drinking fluid. At 4, 6, and 12 weeks, the oral glucose tolerance test (OGTT) was conducted in fasting rats. Green tea supplementation was found to decrease plasma glucose levels and plasma insulin levels at week 4 and 6 but not only plasma insulin at week 12. The lack of effect at week 12 on blood glucose is interesting and deserved further study. In addition to improved glucose and insulin homeostasis, the green tea group also had lower fasting plasma triacylglyceride and free fatty acids than the control rats.

Many studies have focused on regulation of the expression of genes involved in glucose uptake and insulin signaling to help explain the mechanisms of antidiabetic activities of green tea polyphenol. Wistar rats given high-fructose diet and 0.1 – 0.2% dietary green tea for 6 weeks had increased GLUT4 mRNA both in the liver and in muscle [59]. An increase of insulin receptor substrate (IRS)2 mRNA levels in the liver and IRS1 in the muscle of rats treated green tea extract was also observed. Previous studies found that the lack of functional insulin receptor substrate (IRS) is the key molecular lesion in hepatic insulin [60]. Qin et al. [61] examined the effects of green tea polyphenols in the same model and found that green tea polyphenols (200 mg/kg BW per day) significantly decreased blood glucose and plasma insulin, triglyceride, total cholesterol, LDL-cholesterol and free fatty acids. They also found a significant reduction in blood glucose, plasma insulin, plasma retinol-binding protein 4, and plasma soluble CD36 (sCD36), which is reported to be a novel marker of insulin resistance and inflammation. Green tea polyphenols also increased the cardiac mRNA levels of IRS1 and IRS2. Additionally cardiac mRNA expression of GLUT1, GLUT4 and glycogen synthase 1 were increased by green tea treatment, whereas glycogen synthase kinase 3 β was decreased. Green tea polyphenols also decreased inflammatory factors including TNF α , IL-1 β , and IL-6 and increased zinc-finger protein 36 (an anti-inflammatory marker).

4. Prevention of hypertension and modulation of plasma cholesterol by green tea polyphenols

Hypertension, also known as high blood pressure, is another condition linked to metabolic syndrome. Tea has been shown to reduce blood pressure and improve endothelial function in animal studies. Endothelial dysfunction is an alteration of endothelial cells, resulting from oxidative stress and impaired vasodilatory response [62]. Both hypertension and perturbed homeostasis of the ratio of HDL-cholesterol and low-density lipoprotein-associated (LDL)-cholesterol are risk factors for cardiovascular disease.

The effect of green tea extract on arterial hypertension in Sprague-Dawley rats was examined (Table 3) [63]. The animals were treated with angiotensin (Ang) II to induce to the development of hypertension. At the end of the 13 day experiment, Ang II treated rats had increased blood pressure and left ventricle mass. Co-treatment with 0.6% green tea extract as the sole source of drinking fluid blunted these increases. Green tea treatment also reduced Ang II-induced increases in plasma hydroperoxides and aortic endothelial expression of hemeoxygenase I and SOD, indicating a decrease in vascular oxidative stress.

A second study by the same group found that 0.6% green tea extract as the sole source of drinking fluid reduced final systolic and diastolic blood pressure by 20% and 24%, respectively in Ang II-treated rats after 14 d [64]. Gene expression studies in the hearts of treated rats showed that green tea extract treatment reduced Ang II induced expression of NAD(P)H oxidase expression and activity compared to Ang II-treated controls. This protein plays a key role in the induction of endothelial oxidative stress by Ang II. Similar decreases in the expression of Akt and extracellular responsive kinase (Erk) 1/2 were observed. Both enzymes are downstream effectors of NAD(P)H oxidase.

Potenza et al. examined the effect of EGCG on spontaneously hypertensive rats (SHR), a model of hypertension, insulin resistance and obesity [65]. SHR were treated for 3 weeks with EGCG (200 mg/kg/d) or enalapril (3 mg/kg/d), an angiotensin converting enzyme inhibitor that is used to treat high blood pressure and congestive heart failure. A significant decrease in systolic blood pressure in both EGCG (15% reduction) and enalapril (20% reduction) treated rats compared to SHR control. Additionally, both compounds significantly enhanced NO-induced *ex vivo* vasorelaxation in mesenteric vascular beds

isolated from SHR. EGCG treatment also significantly decreased myocardial infarct size by 30% and improved cardiac function of SHR hearts exposed to ischemia-reperfusion injury. Mechanistic studies suggest that EGCG acts by acutely enhancing NO signaling via the phosphatidylinositol-3-kinase pathway.

By contrast, a second study in another model of spontaneous hypertension, the malignant stroke-prone spontaneously hypertensive rats (M-SHRSP), found that treatment with Polyphenon E (0.5% in the drinking fluid) for 10 wk had no effect on blood pressure, but did significantly delay onset of stroke, compared to control rats [66]. The lack of effect on blood pressure may be due to difference in this model from the SHR model, differences in dose, or some other factor.

Treatment of a type 2 diabetes rat model, the Otsuka Long-Evans Tokushima Fatty rat, with 30 mg/kg/d tea catechins for 12 weeks was shown to improve endothelial function [67]. Systolic blood pressure was reduced by 10% compared to saline-treated control rats. Catechin-treated rats also exhibited increased vasodilation in response to sodium nitroprusside treatment. These effects appear to correlate with decreased NADH oxidase expression and activity.

Green tea preparations have been shown to induce vasodilation *in vitro*. Using rat aortic rings, Lorenz et al. have shown that EGCG can mediate dose-dependent vasodilation [68]. These effects were abrogated by pre-treatment with Ng-nitro L-arginine methyl ester, an inhibitor of endothelial nitric oxide synthase (eNOS). On a molecular level, EGCG induced activation of Erk1/2 and Akt, and increased eNOS phosphorylation.

Green tea and green tea polyphenols have been shown to modulate plasma and tissue levels of both HDL- and LDL-cholesterol. A study in cholesterol-fed New Zealand Rabbits showed that green tea have potential anti-atherosclerotic effects [69]. Rabbits were supplemented with 3 g/L green tea (28% catechins and 10% EGCG by weight) as the sole source of drinking fluid for 21 weeks and a 31% reduction in the formation of aortic atherosclerotic lesions compared to water-treated controls. Green tea supplementation increased the lag phase for low-density lipoprotein oxidation in an *ex vivo* assay with the same rabbits.

Yang and Koo examined the effect of green tea supplementation on serum cholesterol levels and enzymes related to cholesterol metabolism [70]. Supplementation of Sprague-Dawley rats fed a hypercholesterolemic diet with 2 or 4% green tea for 8 weeks dose-dependently reduced total serum cholesterol (17 – 23%) and increase plasma HDL (19 – 59%) compared hypercholesterolemic diet-fed controls. Although there were no changes in the activities of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, cholesterol 7 α -hydroxylase or FAS, the authors did report an increase in fecal bile acid and cholesterol levels. These results indicate that green tea can increase cholesterol excretion and elimination.

This study is somewhat contradictory to results reported by Bursill et al. which showed that supplementation of high cholesterol-fed New Zealand White Rabbits with 0.5 – 2% green tea catechins dose-dependently reduced total plasma cholesterol (60% reduction at 2% green tea catechins), plasma LDL (80% reduction at 2% green tea catechins) [71]. Similarly, liver and aortic cholesterol levels were reduced by 25%. These changes correlated with a decrease in cholesterol synthesis (60% reduction at 2% green tea catechins) and an increase in hepatic LDL receptor activity (80% increase at 2% green tea catechins) and expression (70% increase at 2% green tea catechins). The authors did not examine the specific cholesterol synthetic enzymes affected, but they did note that no change was observed in intestinal absorption of cholesterol. The differences in the results of these two studies may be the

result of the use of different tea preparations or doses. More work is needed to resolve these issues.

In vitro mechanistic studies on tea polyphenols and prevention of cardiovascular disease have focused largely on the antioxidant activity of the compounds and their ability to improve endothelial function. Numerous investigators have reported that tea polyphenols can prevent the oxidation of LDL cholesterol *in vitro* [72-74]. For example, 1 – 10 µg/mL green tea extract was shown to dose-dependently reduce LDL oxidation induced by umbilical vascular endothelial cells [75]. A 61% reduction in LDL oxidation was observed following treatment with 10 µg/mL green tea extract. As mentioned in the previous section, some studies in animal models have confirmed these *in vitro* findings whereas others have not. Further clinical studies on the role of antioxidative activity of tea polyphenols in the prevention of cardiovascular disease are warranted.

5. Prevention of obesity-related fatty liver disease by green tea polyphenols

Hepatic steatosis (fatty liver) is a condition that is defined by fat accumulation within hepatocytes that exceeds 5% of the liver by weight [76]. Initially, it was believed that this condition was mainly attributable to excess alcohol consumption, but studies in the last several decades have also linked obesity and diabetes to the presence of fatty liver [77]. Typically, fatty liver disease related to etiological factors other than alcohol is referred to as non-alcoholic fatty liver disease (NAFLD). In order to distinguish obesity as the driver of steatosis from other causes, we suggest the term obesity-related fatty liver disease (ORFLD). There are several proposed grades of ORFLD that characterize the severity of the condition. Grades 1-2 describe simple steatosis, with mild inflammation, whereas grades 3-4 describe florid steatosis, chronic and acute inflammation throughout the liver, and fibrosis [78]. Currently, NAFLD is the most common form of liver disease [79,80]. It is now widely accepted that NAFLD is the hepatic component of the metabolic syndrome; risk factors for the disease include obesity, insulin resistance, and hypertriglyceridemia [3-5].

Several studies that have reported the effect of tea or tea catechins on hepatic steatosis in animal models. For example, tea catechins have been shown to reduced hepatic steatosis and liver toxicity (as measured by elevated plasma ALT) in rodents treated with ethanol [81-83], tamoxifen [84], endotoxins [85], and liver ischemia/reperfusion injury [86]. Fewer studies, however, have examined the effect of tea constituents on high-fat diet-induced liver pathologies. Murase et al. showed that 0.5% green tea extract in the diet for 11 monthssignificantly decreased liver lipid accumulation caused by a high-fat diet in C57BL/6J mice [30]. Another study showed that treatment with 0.2-0.4% tea catechins for 35 weeks significantly decreased inflammation due to fatty liver in LDL-receptor deficient mice on a hypercholesterolemic diet [87]. Bose et al. [32] have demonstrated that 0.32% dietary EGCG can ameliorate high fat-diet induced ORFLD in C57BL/6J mice. EGCG treatment reduced incidence of hepatic steatosis, liver size (22% decrease), liver triglycerides (69% decrease), and plasma ALT concentration (67% decrease) compared to high fat-fed control mice after 16 weeks.

Accumulation of lipid in the liver can be caused by several pathologies, including an increased availability of free fatty acids for uptake, deregulation of fatty acid oxidation, or increases in *de novo* lipogenesis [88]. Dysfunction in lipoprotein metabolism may also play a role in the development of hepatic steatosis. Treatments with tea in animal models have shown to modulate several of these conditions (see previous sections). More studies need to be conducted on the specific components of green tea that mediate its benefits on liver function, and the underlying mechanisms of action. For example, EGCG and other catechins

decrease fatty acid synthase in cells and cell-free studies [89-91], but effects by specific tea catechins need to be verified *in vivo*.

6. Concluding Remarks

The complex of conditions which make up MetS are a growing public health issue that carry an enormous potential economic burden. For example, it has been estimated that medical spending related to obesity in the United States in 2006 was approximately \$119 billion [92]. Although effective surgical and pharmacological methods have been developed to treat symptoms related to metabolic syndrome, these treatments can be costly and are not without potential adverse effects [93-96]. The development of dietary agents for the prevention or treatment of one or more of the symptoms of MetS, alone or in combination with lifestyle changes and pharmaceutical agents, could represent a cost-effective and safe approach to the problem.

Green tea is already a popular beverage that could be easily deployed as a part of the diet designed to mitigate or prevent the symptoms of MetS. Although a considerable amount of laboratory research has been conducted to demonstrate the efficacy of green tea as a preventive agent for MetS and to understand the underlying mechanisms of action, many questions remain.

As we have discussed in this review, the green tea catechins appear to have activity against MetS, but caffeine may also play a role. At this point, the interactions between the catechins and caffeine with regard to prevention of MetS are poorly understood. Given the stimulatory effects of caffeine, it would be interesting to know how this affects hypertension parameters. Is it possible that caffeine contributes to beneficial effects related to obesity, but worsens hypertension [97]? How can these differential effects be reconciled to maximize benefit while keeping adverse events to a minimum.

A number of potential mechanisms have been proposed to account for the preventive effects of green tea against symptoms of MetS. These include modulation of lipid absorption and metabolism, enhancement of glucose uptake and utilization, antioxidative activity, and others. Given the extent to which tea catechins bind non-specifically to proteins, it is like that they work through multiple mechanisms of action to exert their preventive effects. Still some of the proposed mechanisms of action are based on *in vitro* studies, and seem to occur only at high concentrations of the test compounds. In the absence of compelling *in vivo* data supporting such mechanisms of action, their relevance *in vivo* remains unclear. Further *in vivo* studies which examine the temporal and dose-response relationships governing individual mechanisms are needed. Presumably, the key mechanisms of action are those that occur at the lowest effective concentration and can be demonstrated earliest in the intervention.

Finally, the growing number of studies demonstrating the potential beneficial effects of green tea with regard to MetS have lead to the development and marketing of a number of green tea based dietary supplements. Sales of green tea based dietary supplements in 2008 through the food, drug, and mass market channel were \$5.5 million (USD) [98]. Although green tea beverage has a long history of safe use in the diet, a more limited number of controlled animal and human studies have been conducted to determine the maximum tolerated dose of green tea components given in alternative formulations such as pills, capsules, etc. No large scale controlled human intervention studies have reported serious adverse effects to date, however a number of observational case reports, as well as laboratory studies have suggested that high doses of green tea polyphenols can cause hepatotoxicity [99,100]. The therapeutic index of these agents must be established, not only when the compounds are delivered via the diet, but also when (as they often are) converted

to a bolus formulation (*e.g.* pill, capsule, tincture). Only with such a complete understanding can the potential benefits of green tea for the prevention of MetS be realized.

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Table 1

Effects of green tea polyphenols on body and adipose tissue weight.[/]

Parameters	Treatment	Dose	Duration	Models	Effects	References
Body weight	GTE	130 mg/d	10 d	Male ZR	↓	[23]
	GTE	2% in diet	14 d	Male SDR	↓	[24]
	GTE	1% in diet	6 wk	<i>ob/ob</i> mice	↓	[25]
	GTC, DGT	1% in diet	23 d	Male SDR	↓	[26]
		2% in diet	6 wk	C57BL/6J	-	[27]
	DGT			<i>ob/ob</i> mice	↓	
	GTC	0.3 % in diet	12 mo	SAMP10 mice	↓	[28]
	EGCG	0.2 – 0.5% in fluid	11 mo	C57BL/6J mice	↓	[29,30]
	EGCG	0.32% in diet	16 wk	C57BL/6J mice	↓	[32]
	EGCG	0.32% in diet	4 wk	C57BL/6J mice	(not sig)	[32]
Adipose tissue Weight	EGCG	0.25 – 1.0% in diet	4 wk	Wistar rats	(not sig)	[34]
	GTE	130 mg/d	10 d	Male ZR	↓	[23]
	GTE	2% in diet	14 d	Male SDR	↓	[24]
	GTE	1% in diet	6 wk	<i>ob/ob</i> mice	↓	[25]
	GTC	0.5% in fluid	8 wk	Male SDR	↓	[31]
	EGCG	0.2 – 0.5% in fluid	11 mo	C57BL/6J mice	↓	[29,30]
	EGCG	0.32% in diet	16 wk	C57BL/6J mice	↓	[32]
	EGCG	0.32% in diet	4 wk	C57BL/6J mice	↓	[32]
	EGCG	1% in diet	1 mo	SDR	↓	[33]

[/] GTC, green tea catechins; GTE, green tea extract; *ob/ob*, leptin-deficient; SDR, Sprague-Dawley rat; ZR, Zucker rat

Table 2

Effects of green tea polyphenols on markers of diabetes.¹

Parameters	Type	Dose	Duration	Models	Effects	References
Blood/plasma glucose	GTE	0.5% in fluid	4 wk	STZ-treated rats	↓	[51]
	GTE	2.0% in fluid	4 wk	STZ-treated rats	↑	[51]
	GTE	1.25% in diet	3 mo	STZ-treated rats	↓	[54]
	GTE	0.5% in fluid	4, 6 wk	SDR (non-diabetic models)	↓	[58]
	GTE	50, 100 mg/kg	4 wk	Wistar Albino rats	↓	[55]
	GTC	200 mg/kg	6 wk	Wistar rats	↓	[61]
	EC	30 mg/kg (bid)	6 d	STZ-treated rats	↓	[53]
	EGCG	0.25 – 1% in diet	7 wk	ZDF rats	↓	[56]
	EGCG	0.5% in diet	11 mo	C57BL/6J mice	↓	[30]
	EGCG	25mg/kg (bid)	8 wk	STZ-treated Wistar	↓	[52]
Plasma insulin	GTE	0.5% in fluid	4, 6 wk	SDR (non-diabetic models)	↓	[58]
	GTC	200 mg/kg	6 wk	Wistar rats	↓	[61]
Insulin sensitivity & Glucose tolerance	GTE	80 mg/kg	12 wk	Beagle dogs	↑	[57]
	GTE	0.5% in fluid	4–12 wk	SDR (non-diabetic models)	↑	[58]
	GTE	0.5 – 2.0% in fluid	4 wk	STZ-treated rats	improved	[51]
	EGCG	0.2 – 1% in diet	7 wk	<i>db/db</i> mice	improved	[56]
HOMA-IR	GTE	80 mg/kg	12 wk	Beagle dogs	↓	[57]

¹ *db/db*, leptin-receptor deficient; GTC, green tea catechins; GTE, green tea extract; SDR, Sprague-Dawley rat; STZ, streptozotocin

Table 3

Effects of green tea polyphenols on markers of blood pressure, cholesterol and triglycerides.[/]

Parameters	Type	Dose	Duration	Models	Effects	References
Blood pressure	GTE	0.6% in fluid	13 d	Ang II-treated SDR	↓	[63]
	GTC	30 mg/kg	12 wk	OLETF rat	↓	[67]
	GTC	0.5% in fluid	10 wk	M-SHRSP	-	[65]
	EGCG	200 mg/kg/	3 wk	SHR	↓	[65]
Plasma TG & cholesterol	GTE	2 – 4% in fluid	8 wk	Male SDR	↓ total cholesterol	[70]
	GTC	2% in diet	4 wk	NZ rabbits	↓ total cholesterol	[71]
	EGCG	0.2 – 0.5% in diet	11 mo	C57BL/6J mice	↓ total cholesterol	[30]
	EGCG	1.0% in diet	4 wk	Wistar rats	↓ total cholesterol	[34]
	EGCG	1% in diet	1 mo	SDR	↓ TG	[33]
	GTC	2% in diet	4 wk	NZ rabbits	↓ LDL	[71]
	EGCG	1.0% in diet	4 wk	Wistar rats	↓ Non-HDL	[34]
	GTE	2 – 4% in fluid	8 wk	Male SDR	↑ HDL	[70]
Others	GTC	2% in diet	4 wk	NZ rabbits	↓ cholesterol synthesis	[71]
	GTC	2% in diet	4 wk	NZ Rabbit	↓ aortic cholesterol	[71]
	EGCG	200 mg/kg	3 wk	SHR	↓ MI size	[65]
	EGCG	200 mg/kg	3 wk	SHR	↑ cardiac function	[65]

[/] GTC, green tea catechins; GTE, green tea extract; HDL, high-density lipoprotein associated cholesterol; LDL, low-density lipoprotein associated cholesterol; M-SHRSP, malignant stroke-prone spontaneously hypertensive rat; NZ, New Zealand; OLETF, Otsuka Long-Evans Tokushima Fatty; SDR, Sprague-Dawley rat; SHR, spontaneously hypertensive rat;