

Green Tea Extract Decreases Oxidative Stress and Improves Insulin Sensitivity in an Animal Model of Insulin Resistance, the Fructose-Fed Rat

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Background: Tea polyphenols, as both insulin potentiating factors and antioxidants, are postulated to act in preventing the metabolic syndrome, which is characterized by insulin resistance, dyslipidemia, and increased oxidative stress.

Objective and Methods: Using an animal model of insulin resistance, our objective was to determine the effects of a green tea extract on oxidative stress parameters and insulin sensitivity. Wistar rats, 10 per group, received a high-fructose diet (FD) for 6 weeks, or the same diet (FD) plus 1 or 2 g of green tea solids/kg diet.

Results: Signs of insulin resistance (hyperglycemia, hypertriglyceridemia, and hyperinsulinemia) developed in rats receiving the FD, but not in those of the control group. In contrast, animals receiving added tea solids exhibited decreases in glycemia, insulinemia, and triglyceridemia, consistent with an insulin-potentiating effect of tea. In parallel, oxidative stress was decreased by tea consumption with lower plasma lipid peroxidation, sulfhydryl (SH) group oxidation, and DNA oxidative damage. In summary, the addition of green tea extracts to the diet, inducing insulin resistance, led to protective effects of green tea against both oxidative stress and insulin resistance.

Conclusions: These data suggest that green tea may be beneficial for people with decreased insulin sensitivity and increased oxidative stress, such as those with the metabolic syndrome or type 2 diabetes.

INTRODUCTION

The metabolic syndrome is a cluster of metabolic abnormalities, with insulin resistance as a major characteristic [1,2]. This nutritional disorder is increasing rapidly and is a leading cause of diabetes and cardiovascular disease. Oxidative stress is linked to insulin resistance, and cardiovascular implications of oxidative stress in insulin-resistant conditions are well documented [3]. This association is not restricted to insulin resistance in type 2 diabetes, but is also evident in patients with the metabolic syndrome [4]. In the metabolic syndrome, a clustering of sources of oxidative stress is related to hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and inadequate antioxidant defenses. Alteration of insulin sensitivity leads to a higher rate of glucose oxidation and increased production of OH [5]. Oxidative stress has been identified as a

key factor in diabetes and atherosclerosis [6–11]. Thus, uncontrolled free radical production might be one of the mechanisms underlying the development of comorbidities in individuals with the metabolic syndrome. For these patients, an increase in dietary antioxidant intake could represent a potential strategy to reduce the incidence of diabetes and cardiovascular disease [12]. Many polyphenols in foods are potent antioxidants [13], and they have been linked with the hypothesis that their redox activities may confer specific health benefits [14]. Polyphenols from tea could be of special interest in the metabolic syndrome because epidemiologic observations and laboratory studies have shown that green tea has a variety of health effects, including antioxidant and hypolipemic activities [15–17]. The most widely known health benefits of tea relate to the polyphenols as the principal active ingredients in protection against oxidative damage, but polyphenols in tea

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also may increase insulin sensitivity and consequently may act in preventing or alleviating diabetes. Antidiabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes have been reported [18]. Tea contains compounds with *in vitro* insulin-enhancing activity [19,20]. The predominant active ingredient seems to be epigallocatechin gallate (EGCG), and we have reported previously that black and green tea extracts and EGCG increase glucose uptake by rat epididymal adipocytes in the presence or absence of added insulin [20].

In rodents, a high-fructose diet (FD) is used widely to induce insulin resistance and the metabolic syndrome [21–23]. Fructose-rich diets stimulate lipogenesis and lead to extrahepatic insulin resistance and high blood pressure [24–26]. Thus, the aim of this study was to investigate, using the fructose-fed rat as an animal model of insulin resistance, the effects of green tea consumption on plasma oxidative stress parameters and their association with potential changes in insulin sensitivity.

MATERIALS AND METHODS

Diets

A fructose-rich diet (FD) was fed to rats [27]. The control group (C) received standard Purina chow for 6 weeks. The diets were purchased from SAFE (89290 Augis, France). The synthetic FD diet contained the following in g/kg: casein, 200; fructose, 600; corn oil, 50; alphacel, 50; DL methionine, 3; choline bitartrate, 2; AIN-76 mineral mix, 35; and AIN-76A vitamin mix, 10 [28]. The green tea solids were obtained from Unilever France (F92842 Rueil Malmaison, France). Green tea polyphenols were quantified by high-performance liquid chromatography (HPLC). They contained 373 mg/g of total polyphenols (127.5 EGCG, 92.4 EGC, 37.3 ECG, 24.0 EC, 59.4 caffeine, and 1.95 L-theanine).

Animals

Forty male Wistar rats (Charles River, L'Arbresle, France), 6 weeks old and weighing roughly 150 g, were housed in wire-bottomed cages in a temperature-controlled room (22°C) with a 12 h light/12 h dark cycle. The rats were maintained and handled according to the Guide for the Care and Use of Laboratory Rats (National Institutes of Health [NIH], 1985). All rats were adapted and fed a standard Purina chow diet for 1 week. They then were randomly divided into 4 groups of 10 rats. The FD group received the FD *ad libitum* for 6 weeks. Two other groups received the same diet for 6 weeks, but it contained either 1 g or 2 g green tea solids/kg diet (FD plus 1 g group) (FD plus 2 g group). The control group received standard Purina chow for 6 weeks. Rats were housed in individual cages and had unrestricted access to food and water.

Food intakes were measured daily, and body weights were measured weekly.

Blood Sampling

After overnight food deprivation, rats were weighed and then anesthetized with sodium pentobarbital given intraperitoneally. Blood was collected by heart puncture in heparinized tubes protected from light and was centrifuged at room temperature for 10 minutes at 3000 g. Plasma was immediately isolated, aliquoted, and stored at -80°C until analyzed. Immediately after blood collection, the rats were sacrificed, and liver was removed, weighed, frozen in liquid nitrogen, and stored until analysis. A total of 500 mg of tissue sample was extracted in buffer (10 mmol/L Tris-NaOH [sodium hydroxide], 1 mmol/L DTPA [diethylenetriaminepentaacetic acid], 1 mmol/L L-PMSF [phenylmethanesulphonylfluoride], pH 7.4) and centrifuged at 3000 g at 4°C for 10 minutes.

Biological Parameters. Insulin was measured by enzyme-linked immunosorbent assay (ELISA) (American Laboratory Products Company, Windham, NJ). Glucose and triglycerides were assessed by enzymatic and colorimetric methods on a Roche/Hitachi Model P (Roche Diagnostics, Indianapolis, IN). Plasma malonaldehyde (MDA) concentrations were assessed using HPLC [30]. Plasma sulfhydryl (SH) groups were assayed as described [31]. The calibration was obtained from a stock solution of 100 mM N-acetyl cysteine (NAC) in the range of 0.125 to 1 mM. Standards and plasma samples were diluted in phosphate buffer 0.05 M, ethylenediaminetetraacetic acid (EDTA) 1 mM, pH 8, and Dtnb-5,5'-dithio-bis (DTNB), 2.5 mM, and absorbance measured at 412 nm. For the comet assay and DNA damage determinations, total blood was stored as described [29]. In all, 500 μl of blood was stabilized with 500 μl of a 20/80 (v/v) mixture of dimethylsulfoxide (DMSO) and RPMI 1640 cell culture medium. Aliquots of this mixture were frozen progressively overnight to -80°C with the use of cryopreservation vessels (Bicell, Fisher Bioblock Scientific, Lyon, France) in a -80°C freezer. After 1 night, samples were transferred from the cryopreservation vessels to storage at -80°C until analyses were performed. DNA damage was evaluated with the comet assay (single-cell gel electrophoresis) on total blood [29]. Results were expressed as tail moment (TEM). Three samples per animals were assayed with 50 cells/sample. The mean of 3 determinations was calculated for each rat.

Statistical Analyses

Statistical analyses of the data were performed by analysis of variance. Individual mean comparisons were identified with Duncan's Multiple Range Test (SAS Institute, Cary, NC). Values were expressed as mean \pm SEM. Statistical significance was set at $p < 0.05$.

Table 1. Food Intakes and Body Weight of Animals

	Control	FD	FD + 1 g Tea Solids/kg Diet	FD + 2 g Tea Solids/kg Diet
Food intake, g/d	21.5 ± 0.6 ^a	20.7 ± 0.8 ^a	20.5 ± 0.9 ^a	20.3 ± 1.5 ^a
Body weight, g	339 ± 6 ^a	360 ± 7 ^a	350 ± 5 ^a	353 ± 7 ^a

^a Numbers in each row with different superscripts are significantly different at $p < 0.05$.

RESULTS

Fructose in the diet did not affect food intake and body weight (Table 1), and green tea solids added to the FD did not change food intake. Body weight tended to be lower when tea solids were added compared with the FD group ($p = 0.08$).

In rats receiving the fructose-rich diet (FD group), plasma insulin, glucose, and triglyceride concentrations were significantly ($p < 0.05$) higher than in the control group. Addition of green tea solids to the FD lowered ($p < 0.001$) plasma fasting glucose (Fig. 1), triglycerides (Fig. 2), and insulin concentrations (Fig. 3).

As shown in Table 2, lipid peroxidation, measured by plasma MDA, was increased in FD rats compared with the control group ($p < 0.05$). Green tea extracts added to the control group did not modify the oxidative stress markers (data not shown). In contrast, FD animals receiving added tea solids exhibited lowered plasma MDA levels compared with FD rats, indicating a lesser extent of lipid peroxidation. DNA oxidative damage, measured by the comet assay, was also decreased. Green tea had a protective effect on protein oxidation based on plasma thiol (SH) groups. In the liver, no significant effects of consumption of green tea extracts based on SH groups and MDA were noted (Table 3). The beneficial effects of tea on oxidative stress and insulin sensitivity were observed at the 2

levels of tea extracts tested, but effects were not enhanced at the higher level of tea consumption.

DISCUSSION

The aim of this study was to investigate, in an animal model of insulin resistance, the effects of consumption of green tea extract on plasma oxidative stress parameters and insulin sensitivity. In rats receiving FD, plasma insulin, glucose, and triglycerides were increased compared with those given the control diet. These data are in agreement with several reports [27,32,33] and confirm that consumption of high-fructose diets leads to the development of insulin resistance. In addition, high levels of dietary fructose have been reported to enhance oxidative damage in rats [33,34]. In the present work, the FD animals exhibited increased levels of oxidative stress parameters, compared with the control group. The underlying mechanisms for the detrimental consequences of a high-fructose diet in animal models are not totally understood. A link between oxidative stress and insulin resistance has been demonstrated [35], and oxidative stress has been proposed as one of the underlying causes of the development of insulin resistance, beta-cell dysfunction, and impaired glucose tolerance [36]. When green tea extracts were added to the fructose

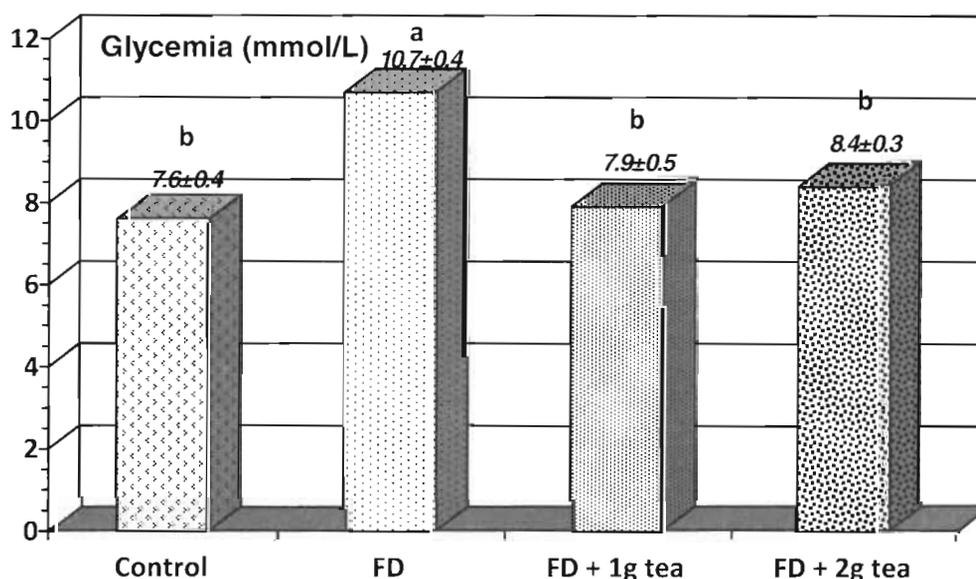


Fig. 1. Effect of green tea extracts on glycemia in FD rats. Numbers with different superscripts are significantly different at $p < 0.05$.

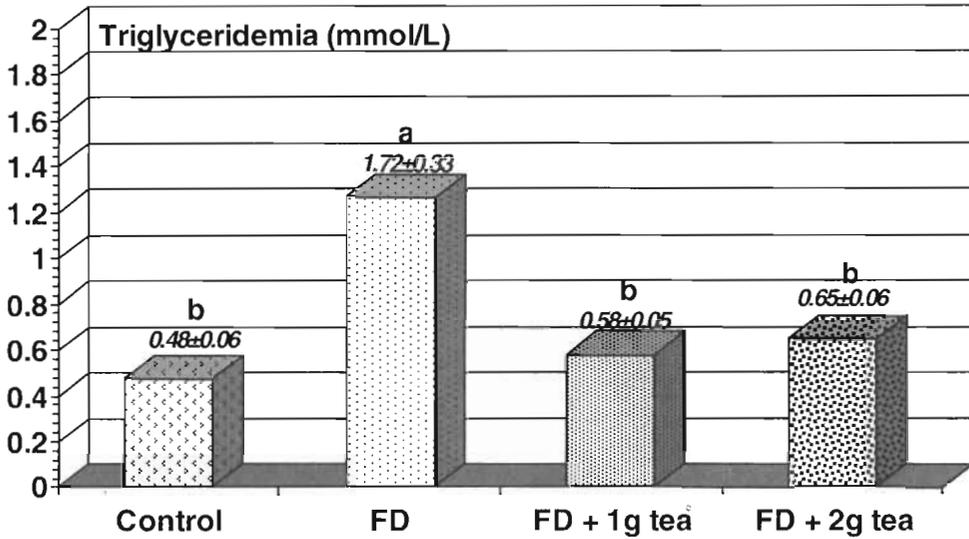


Fig. 2. Effect of green tea extracts on triglyceridemia in FD rats. Numbers with different superscripts are significantly different at $p < 0.05$.

diet, fasting glucose, insulin, and triglycerides all decreased. These data are consistent with the insulin-enhancing activity of green tea that we observed previously *in vitro* [20]. In rats fed a high-fructose diet, we reported that green tea extracts regulate the expression of genes involved in glucose uptake and insulin signaling [37]. The effect of green tea extracts on glycemic control has also been observed in other studies in man [38] and in animals [38,39]. Because glycemic control is implicated in preventing cardiovascular disease [40], this effect of tea consumption could be predictive of benefits for people at risk of developing cardiovascular disease.

In association with the insulin-enhancing activity of tea, this study supports the antioxidant effects of green tea. Antioxidant activities of green tea polyphenols are well

documented [41,42] and have been attributed mainly to EGCG [43], which is particularly high in green tea. EGCG can act as an antioxidant by trapping oxygen reactive species, inhibiting lipid peroxidation, and protecting cell membranes against oxidative stress [44]. Green tea extracts in the diet decreased plasma lipid peroxidation. Other studies, consistent with our data, reported the beneficial effects of green tea consumption on lipoprotein oxidation [45] and lipoperoxide metabolism [46]. It is well documented that decreasing lipid peroxidation is an important health challenge to avoid oxidative damage of the arterial walls, and the lowering effects of tea on lipid peroxidation suggest possible benefit for patients with type 2 diabetes who have been given a diagnosis of cardiovascular disease.

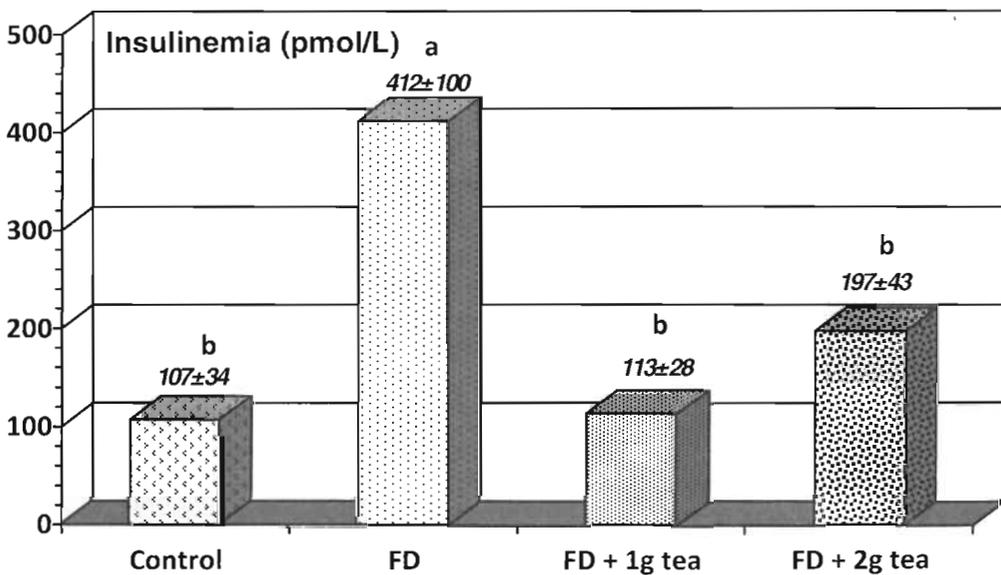


Fig. 3. Effect of green tea extracts on fasting insulinemia in FD rats. Numbers with different superscripts are significantly different at $p < 0.05$.

Table 2. Effects of Green Tea Extracts on Plasma Oxidative Stress Parameters

Plasma Parameters	Group I Fructose Diet (FD)	Group II FD + 1 g Tea Solid/kg	Group III FD + 2 g Tea Solid/kg	Control
MDA, $\mu\text{mol/L}$	4.75 ± 0.28^a	3.77 ± 0.25^b	4.02 ± 0.38^a	3.5 ± 0.25^b
SH groups, $\mu\text{mol/L}$	5.4 ± 0.05^b	6.0 ± 0.15^d	5.8 ± 0.05^a	6.1 ± 0.15^a
DNA damage ¹ , Comet (TEM)	12.8 ± 0.75^a	8.1 ± 0.61^c	10.8 ± 0.58^b	7.60 ± 0.50^c

^a Numbers in each row with different superscripts are significantly different at $p < 0.05$.

^b Numbers in each row with similar superscripts are not significantly different at $p < 0.05$.

¹ Arbitrary units.

We also observed that green tea extracts decreased oxidative DNA damage, as assessed by the comet assay. In agreement with these data, another study showed that green tea catechins partially protect DNA from OH radical-induced strand breaks and base damage through fast chemical repair [47]. In heavy smokers [48] at high risk for oxidative stress, a beneficial effect of a 4-month tea intervention on oxidative damage has been reported. The increase in plasma SH groups, whose oxidation is an early determinant of oxidative stress [49], indicates protective effects of green tea against free radicals and glucose-mediated protein damage. These data are consistent with the protective activity of green tea extracts against protein oxidation and glycation [50]. Protein oxidation has been reported as a risk factor for oxidative complications like nephropathies and glomerulopathies in diabetes [51].

In contrast to plasma parameters, no significant changes in lipid or protein oxidation were noted in the liver of animals receiving green tea extracts. However, antioxidant effects of tea consumption have been reported in the liver of rats fed diets containing 3% green tea leaf powder [52], leading to decreased levels of liver peroxides. The lower levels of green tea extracts (0.1% and 0.2%) used in our study could possibly explain this discrepancy.

This study reports the association between antioxidant and insulin-enhancing properties of green tea extracts. Mechanisms for the induction of insulin resistance by oxidative stress have been proposed [35,36], and, conversely, high insulin is a leading cause of oxidative stress [3]. In this study, the observed improvement in insulin sensitivity could be part of the antioxidant effects of green tea, because previous studies in fructose-fed rats have reported that a free radical scavenger, like vitamin E, improved insulin sensitivity [53]. Conversely, the insulin-potentiating effects of green tea could contribute to

the antioxidant effects. Similarly, an insulin sensitizer, metformin, has been shown to improve the free radical defense system [54].

The beneficial effects of the green tea at 2 g/kg diet were not greater than those observed in animals consuming 1 g of tea solids/kg diet. We also observed similar effects of 1 and 2 g of tea solids added per kg of diet in the expression of genes involved in glucose uptake and insulin signaling [37]. The reasons for this lack of a dose response are under study.

Finally, in the present study, trends showed that body weight decreased in animals consuming the high-fructose diet plus tea solids. This observation is consistent with previous studies indicating that green tea catechins contribute to body weight regulation by increasing fat oxidation and energy expenditure in experimental animals [55] and humans [56].

These preliminary data are encouraging and suggest a possible association between green tea extract consumption and protective effects against insulin resistance and oxidative stress. Because green tea extracts were added to the FD at the beginning of the experiment and were not administered to animals that had already developed the metabolic syndrome, the effects observed in the present study should be reported more as preventing oxidative stress and insulin resistance than as reducing effects on oxidative stress induced by the diet. Additional studies are needed to determine whether green tea consumption represents a viable nutritional approach in individuals at increased risk for high levels of oxidative stress and insulin resistance.

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Table 3. Effects of Green Tea Extracts on Oxidative Stress Parameters in Liver

Liver Parameters	Group I Fructose Diet (FD)	Group II FD + 1 g Tea Solid/kg	Group III FD + 2 g Tea Solid/kg	Control Group
MDA, $\mu\text{mol/g prot}$	0.36 ± 0.02	0.36 ± 0.02	0.39 ± 0.02	0.38 ± 0.02
SH groups, $\mu\text{mol/g prot}$	62.7 ± 3.9	57.9 ± 3.9	65.1 ± 5.5	60 ± 4.9

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