Effect of Green Tea Supplementation on Insulin Sensitivity in Sprague–Dawley Rats

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Epidemiological observations and laboratory studies have shown that green tea has a variety of health effects, including antitumor, antioxidative, and hypolipidemic activities. The aim of this study was to examine whether it had an effect on glucose tolerance and insulin sensitivity in Sprague–Dawley rats. In experiment 1 (in vivo study), rats were divided into two groups: a control group fed standard chow and deionized distilled water and a "green tea" group fed the same chow diet but with green tea instead of water (0.5 g of lyophilized green tea powder dissolved in 100 mL of deionized distilled water). After 12 weeks of green tea supplementation, the green tea group had lower fasting plasma levels of glucose, insulin, triglyceride, and free fatty acid than the control rats. Insulin-stimulated glucose uptake of, and insulin binding to, adipocytes were significantly increased in the green tea group. In experiment 2 (in vitro study), a tea polyphenol extract was used to determine its effect on insulin activity in vitro. Green tea polyphenols (0.075%) significantly increased basal and insulin-stimulated glucose uptake of adipocytes. Results demonstrated that green tea increases insulin sensitivity in Sprague–Dawley rats and that green tea polyphenol is one of the active components.

KEYWORDS: Green tea; insulin sensitivity; diabetes; polyphenol; triglyceride

INTRODUCTION

Tea is a popular beverage worldwide, and tea drinking has been a part of Chinese culture for the past 5000 years. Many kinds of tea are served as drinks, including unfermented green tea, semifermented oolong and pou-chong tea, completely fermented black tea, and postfermented pu-erh tea.

Green tea has been reported to have various biological activities including antifungal (*1*, *2*), anticariogenic (*3*, *4*), antitumor (*5*, *6*), antioxidative (*7*, *8*), and cholesterol-lowering (*9*, *10*) effects. Panzram (*11*) found that patients with non-insulin-dependent diabetes mellitus have higher cardiovascular morbidity and mortality than normal subjects. Reaven (*12*) also pointed out the risk factors of cardiovascular disease including hyperinsulinemia, insulin resistance, glucose intolerance, and hypertension and called them collectively "Syndrome X". Tea has been shown to delay the oxidation of low-density lipoprotein (LDL) in vitro (*13*), in animal studies (*14*) and in human subjects (*15*), and thus could help prevent cardiovascular disease.

The relationship between tea drinking and plasma glucose homeostasis was confusing. Plasma glucose levels in alloxan-induced diabetic rats were lowered when the animals were given (−)-epicatechin (*16*, *17*), which is also found in tea. Gomes et al. (*18*) found that aqueous extracts of green tea and black tea have a hypoglycemic effect in streptozotocin (STZ)-induced diabetic rats. In addition, tea polyphenols inhibit the activity of α-amylase and sucrase in the small intestine, interfere with the digestion of carbohydrates, and inhibit the increase in plasma glucose levels in rats fed starch or sucrose (*19*). However, no hypoglycemic effect was found when (−)-epicatechin was given to STZ-induced diabetic rats and spontaneously diabetic BB/E rats (*20*).

Polyphenolic metabolites from fruits, vegetables, and tea are common components of the human diet. They have been shown to act as strong antioxidants in various systems, exhibiting many biological activities (*21*, *22*). Among commercial tea varieties, green tea is an unfermented product containing a relatively large amount of polyphenol compared to semifermented and completely fermented teas. Previous study has shown that green tea enhances in vitro insulin activity, and the primary active component in green tea was suggested to be epigallocatechin gallate (*23*). Because in vitro study has shown that green tea potentiated insulin activity, long-term in vivo experiments are needed to further realize the changes of blood glucose and insulin levels following green tea consumption. The present study was therefore designed to evaluate the effect of green tea

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supplementation on glucose tolerance and insulin sensitivity in rats. Moreover, to determine whether tea polyphenol was an active component, adipocytes from rats were treated with tea polyphenol extract and the effect on glucose uptake was observed.

MATERIALS AND METHODS

Preparation of Green Tea and the Polyphenolic Fraction. Green tea, which was manufactured from the same batch of summer tea leaves of TTES No. 12 variety, was provided by the Taiwan Tea Experiment Station. It was ground to a powder and passed through a 30 mesh sieve. Tea infusion was prepared according to the standard method of the Taiwan Tea Experiment Station. Ten grams of tea powder was soaked for 5 min in 500 mL of water at 100 °C and then filtered. The filtrate was freeze-dried, and the lyophilized powder (2.5 g) was stored in a desiccator. The freeze-dried powder of green tea extract contained epicatechin, 18.53 mg·g^{-1}; epicatechin gallate, 21.51 mg·g^{-1}; epigallocatechin, 57.87 mg·g^{-1}; and epigallocatechin gallate, 199.49 mg·g^{-1}. For animal trials, the lyophilized powder (0.5 g) was dissolved in 100 mL of deionized distilled water.

To extract green tea polyphenols, tea powder was dissolved in water as above and filtered, and then the filtrate was extracted with an equal volume of chloroform to remove caffeine and pigments. The aqueous layer was extracted with an equal volume of ethyl acetate as described previously (*24*), and the polyphenol-containing ethyl acetate layer was evaporated and freeze-dried. The lyophilized polyphenol powder (0.75 g) was dissolved in 100 mL of deionized distilled water for the in vitro study of the glucose uptake of adipocytes.

Animal Experiment. Male Sprague−Dawley (SD) rats weighing 200−250 g were housed three in each cage in an air-conditioned room (22 ± 2 °C) with a 12 h light cycle (6:00 a.m.−6:00 p.m.). Animals were maintained according to the guidelines established in the *Taiwan Government Guide for the Care and Use of Laboratory Animals*. In experiment 1 (in vivo study), all rats were fed standard rat chow (Purina, St. Louis, MO) composed of 60% vegetable starch, 12% fat, and 28% protein. They were randomly divided into two groups ($n = 8$): a control (C) group given water to drink and a green tea (G) group given green tea instead of water; fresh drink was provided at 6:00 p.m. each day. The experiment lasted 12 weeks. Various parameters were measured over the 12 weeks, as detailed below, then the animals were killed at the end of the experiment, and relevant plasma levels were measured and adipocytes prepared.

Oral Glucose Tolerance Test (OGTT). After 4, 6, and 12 weeks, the rats were subjected to an OGTT following the procedure described by Whittington et al. (*25*). After overnight fasting, a 0 h blood sample (0.5 mL) was taken by cutting the tail tip, then a glucose solution (2 g/mL) was immediately administered by gavage [2 g/kg of body weight (BW)], and four more tail vein blood samples were taken at 30, 60, 90, and 120 min after glucose administration. All blood samples were collected in Eppendorf tubes prerinsed with heparin solution (20 IU/mL) and were kept on ice until centrifuged (3500*g* at 4 °C for 30 min) to separate the plasma. The plasma samples were frozen at −20 °C until assayed for glucose and insulin. The plasma glucose concentration was measured using a glucose analyzer (model 2300; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin levels were determined by a radioimmunoassay technique developed in our laboratory (*26*), which can be used to assay both human and rat insulin.

Isolation of Adipocytes. At the end of the 12 weeks of animal experiment, the rats were killed by decapitation after overnight fasting, and blood was collected in heparinized tubes. The plasma was separated by centrifugation and stored at − 20 °C until assayed for triglyceride (TG), free fatty acid (FFA), glucose, and insulin. Measurements of plasma glucose and insulin are descried above. Plasma triglyceride (TG) levels were analyzed using a commercial enzymatic kit from E. Merk (Darmstadt, Germany). Plasma free fatty acid (FFA) levels were determined using colorimetric methods (*27*). The epididymal fat pads excised from each group of rats were pooled to isolate adipocytes using the Rodbell method (*28*) with minor modifications. Fat pads from two to three rats were pooled for each sample of test. Briefly, the fat tissue was minced and incubated for 1 h at 37 °C in Krebs−Ringer bicarbonate

Table 1. Body Weight, Food Intake, Water/Tea Intake, and Urine Output in Rats at 12 Weeks with or without Green Tea Supplementation[a]

	control	green tea
body weight (g/day)	512 ± 26	505 ± 29
food intake (g/day)	30.2 ± 6.3	34.6 ± 3.6
water/tea intake (mL/day)	39.2 ± 10.8	37.5 ± 9.5
urine (mL/day)	27.3 ± 8.9	25.2 ± 4.6

[a] Values are shown as the mean ± SD. There was no significant difference between the control and green tea groups ($P > 0.05$).

(KRB) buffer solution containing 1% bovine serum albumin (KRBB) and 0.1% collagenase in an oxygen-rich shaking chamber (CO$_2$/O$_2$, 5:95; 75 strokes/min). The suspension was then filtered through nylon mesh (400 μm) and centrifuged at 100 rpm for 1 min. The supernatant containing the adipocytes was harvested, and the cells were washed twice with, and resuspended, in KRBB. The number of cells in the adipocytes suspension was determined following fixation with 2% osmium tetraoxide. The lipocrit was checked and used for normalization of the fat cell number before, during, and after each experiment.

Insulin Binding to Adipocytes. Binding of insulin to adipocytes was performed as described previously (*29*). Briefly, 50 μL of [^{125}I]-insulin (final concentration = 0.25 nmol/L) and 50 μL of increasing concentrations of unlabeled insulin (none or final concentrations of 1 pM−1 μM) were mixed and added to 400 μL aliquots of the adipocyte suspension (2 × 10^5 cells). The mixture was incubated for 30 min in a 95% oxygen chamber at 37 °C with gentle shaking (75 strokes/min), and then 300 μL of the cell suspension was transferred to a new centrifuge tube containing 200 μL of silicon oil. The mixture was centrifuged at 1000*g* at room temperature for 1.5 min, and then the cellular layer was transferred to a vial containing 4 mL of a cocktail for the counting of radioactivity using a liquid scintillation counter. A Scatchard plot was used to determine the number of insulin-binding sites (B_{max}) and the binding affinity (K_d) of the cells.

Glucose Uptake of Adipocytes. Insulin-stimulated glucose uptake of adipocytes was determined by measuring the transport of 2-deoxy-glucose (2-DG) into the cells, as described by Garvey et al. (*30*), with some modifications. Briefly, 400 μL of fat cell suspension was mixed with an increasing concentration of insulin (none or final concentrations of 1 pM−100 nM, 50 μL) and incubated as above for 30 min. Fifty microliters of [^{3}H]2-DG (final concentration = 50 μM) was added, and incubation was continued for another 3 min. The reaction was terminated by adding 200 μL of unlabeled 2-DG (final concentration = 0.14 M) to the mixture, and then 300 μL of the cell suspension was transferred to a new vial containing 200 μL of silicon oil and processed as described for the insulin-binding assay.

In Vitro Study of the Effect of Green Tea Polyphenols on Adipocytes. In experiment 2, adipocytes from 10 male SD rats weighting 350 g were isolated from the epididymal fat pads of normal SD rats as described above, and the assay below was performed at 37 °C. A 400 μL aliquot of the adipocyte suspension was mixed with 50 μL of 0.75% green tea polyphenols (final concentration = 0.075%) for 30 min and then underwent the same procedures as described in the glucose uptake assay.

Statistical Analysis. Data were expressed as the mean ± SD and compared using Student's *t* test. Statistical analysis was performed using a computer program provided in the Microsoft Excel kit (GreyMatter International, Cambridge, MA). Differences between the two groups were considered to be statistically significant when the *P* value was <0.05.

RESULTS

Experiment 1 (in Vivo Study). Body weight, monitored weekly for 12 weeks, showed no significant difference at any time point between the green tea supplemented and the control groups (data not shown). After 12 weeks, no differences between the two groups were seen in the amount of food and liquid intake or in urine production (**Table 1**).
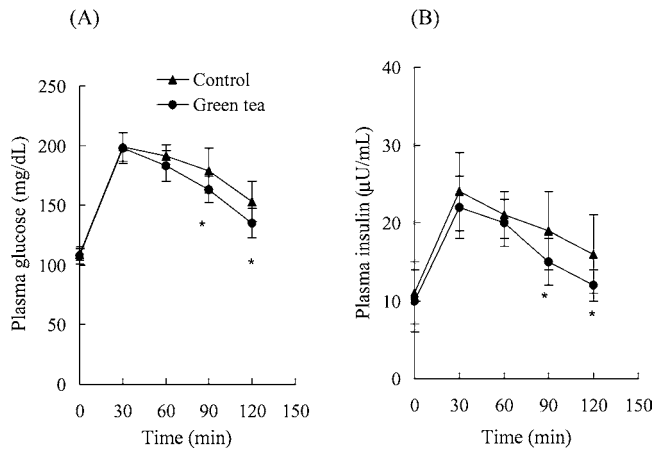
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Figure 1. Changes in plasma levels of glucose (**A**) and insulin (**B**) in rats in response to an oral glucose tolerance test (2 g of glucose/kg of BW) performed after 4 weeks with or without green tea supplementation. Values are shown as the mean ± SD. *, $P < 0.05$ compared to the control group.
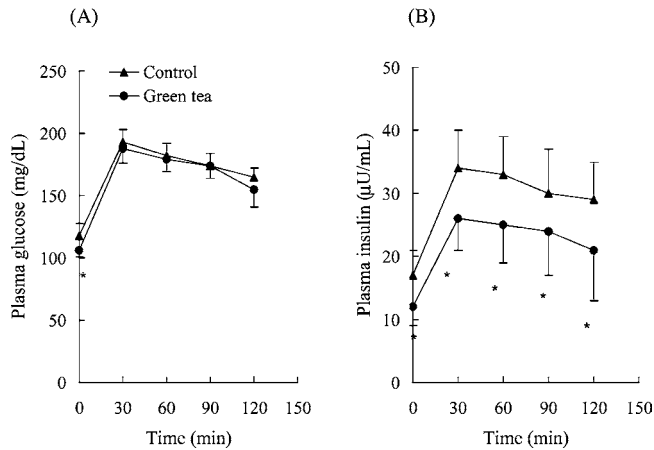

Figure 2. Changes in plasma levels of glucose (**A**) and insulin (**B**) in rats in response to an oral glucose tolerance test (2 g of glucose/kg of BW) performed after 12 weeks with or without green tea supplementation. Values are shown as the mean ± SD. *, $P < 0.05$ compared to the control group.

Table 2. Epididymal Fat Pad Weight, Relative Epididymal Fat Pad Weight, and Fasting Plasma Glucose, Insulin, Free Fatty Acid, and Triglyceride Concentrations in Rats after 12 Weeks with or without Green Tea Supplementation[a]

	control	green tea
epididymal fat pad weight (g)	4.6 ± 1.3	3.7 ± 1.1
relative epididymal fat pad weight (g/100 g of BW)	0.90 ± 0.24	0.73 ± 0.22
fasting plasma glucose (mg/dL)	113 ± 3	105 ± 6*
fasting plasma insulin (μU/mL)	13.4 ± 3.3	9.6 ± 3.6*
fasting plasma free fatty acid (μM)	222 ± 10	154 ± 46*
fasting plasma triglyceride (mg/dL)	69.8 ± 14.9	33.7 ± 12.0*

[a] Values are shown as the mean± SD. *, $P < 0.05$ compared to the control group.
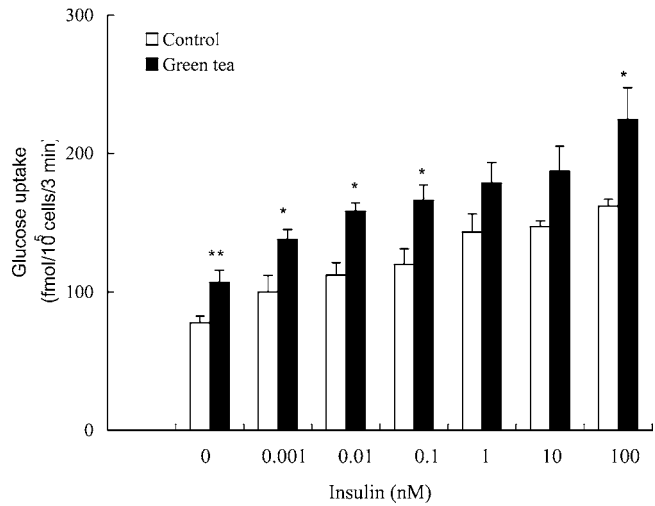

Figure 3. Glucose uptake of adipocytes from rats after 12 weeks with or without green tea supplementation. Values are shown as the mean ± SD ($n = 3$). *, $P < 0.05$ compared to the control group; **, $P < 0.01$ compared to the control group.

After 4 weeks of green tea supplementation, an OGTT showed no significant difference between the two groups in fasting plasma glucose levels, but, during the 2 h interval after glucose ingestion, plasma glucose levels were significantly lower in the green tea group than in the control group at 90 and 120 min after ingestion (**Figure 1A**). Similar results were present for the plasma insulin level (**Figure 1B**). The total areas under the curve (AUC) between 0 and 120 min, representing the magnitude of the glucose and insulin response, were 350 ± 19 mg·h·dL^{-1} and 39 ± 7 μU·h·mL^{-1} for plasma glucose and insulin in the control group, respectively, and 333 ± 17 mg·h·dL^{-1} and 34 ± 4 μU·h·mL^{-1} in the green tea group, respectively. There was no significant difference between the two groups ($P = 0.055$ for glucose response; $P = 0.077$ for insulin response). After 6 weeks of green tea supplementation, the results were similar to those from 4 weeks. However, at 90 min there was no significant difference between the insulin levels in the two groups (data not shown).

After 12 weeks of green tea supplementation, the fasting plasma glucose and insulin levels in the green tea group were significantly lower than those in the control group(**Figure 2**). During the 2 h following glucose ingestion, no difference was seen in the plasma glucose levels between the two groups; however, the plasma insulin levels at all time points were significantly lower than those in the control group. The AUCs for plasma glucose and insulin were 346 ± 13 mg·h·dL^{-1} and 60 ± 18 μU·h·mL^{-1} in the control group, respectively, and 336 ± 11 mg·h·dL^{-1} and 38 ± 10 μU·h·mL^{-1} in the green tea group, respectively. No significant difference was found between the two groups in the AUC for plasma glucose ($P = 0.260$), but the AUC for insulin in the green tea group was significantly lower ($P = 0.004$) than that in the control group, showing that the green tea group had increased insulin sensitivity.

When the animals were killed at the end of the experiment, the fasting plasma glucose and insulin levels were again lower in the green tea group than these in the control group, and, in addition, plasma FFA and TG levels in the green tea group were also significantly lower than those in the control group ($P < 0.05$) (**Table 2**).

Glucose uptake of adipocytes is shown in **Figure 3**. Insulin stimulated adipocyte glucose uptake in a dose-dependent manner. Basal glucose uptake in the green tea group was significantly higher than that in the control group (107 ± 6 vs 78 ± 4 fmol/10^{5}cells/3 min; $P = 0.002$). In the insulin-stimulated glucose uptake, the green tea group also showed a higher value than the control group.

The insulin-binding results for adipocytes (**Figure 4**) provided additional evidence for the beneficial effects of green tea. Insulin binding in the green tea group was significantly higher than
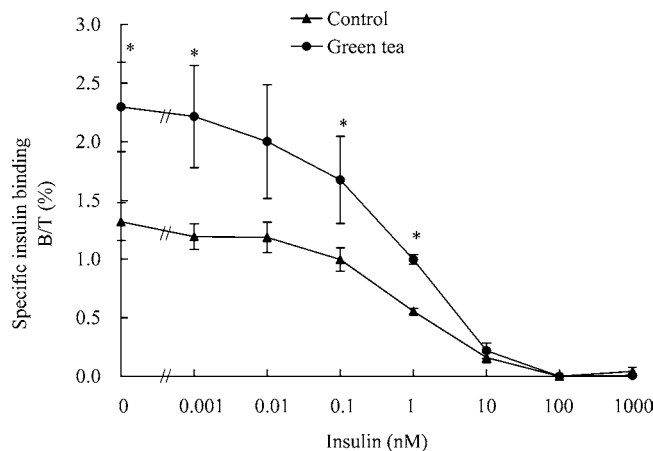
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Figure 4. Insulin binding by adipocytes from rats after 12 weeks with or without green tea supplementation. Values are shown as the mean ± SD ($n = 3$). *, $P < 0.05$ compared to the control group.

Table 3. Insulin Binding Kinetics of Adipocytes from Rats after 12 Weeks with or without Green Tea Supplementation[a]

	high-affinity binding site		low-affinity binding site	
group	Kd (pmol/L)	B_{max} (fmol/ 10^5 cells)	Kd (pmol/L)	B_{max} (fmol/ 10^5 cells)
control	357 ± 11	2.54 ± 0.20	2087 ± 959	9.83 ± 3.47
green tea	280 ± 29	3.56 ± 0.35*	1592 ± 817	13.41 ± 4.71

[a] Values are shown as the mean ± SD ($n = 3$). *, $P < 0.05$ compared to the control group.

that in the control group ($P < 0.05$). Maximum binding was 2.30 ± 0.66% for the green tea group and 1.32 ± 0.28% for the control group. A Scatchard plot of the insulin-displacing curves gave the binding site and affinity results listed in **Table 3**. Green tea supplementation led to a nonsignificant increase in the affinity (decreased K_d) and a significant increase in the number of binding sites (increased B_{max}) for the high-affinity insulin receptor, but no significant difference for the low-affinity insulin receptor. The glucose uptake and insulin competitive binding results therefore showed that the insulin sensitivity of adipocytes from animals given green tea supplementation increased as a result of an increase in the number of high-affinity insulin binding sites in the cells.

Experiment 2 (in Vitro Study). The effect of green tea polyphenols on adipocyte basal glucose uptake and insulin-stimulated (1 nM) glucose uptake was then examined in order to locate the active components of green tea extract. As shown in **Figure 5**, incubation of adipocytes from rats with green tea polyphenols resulted in a significant increase in both activities.

DISCUSSION

It is well-known that obesity causes insulin sensitivity to decrease (*31*). Some research has shown that the body weight of animals given green tea supplementation decreased significantly (*9*, *32*). However, in our study, food and liquid intake was not affected by green tea supplementation. The different results obtained in these studies might be due to the concentration of green tea used. In the above studies, the green tea supplement represented ∼4% of the total weight of the diet (*9*). In our study, green tea drink was given as 0.5 g of tea extract/ 100 mL of deionized distilled water concentration, which accounted for <1% of the diet and may therefore not have affected growth.
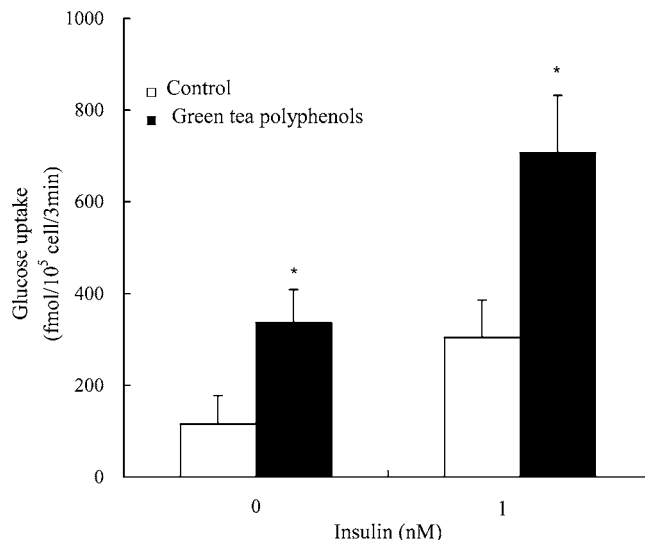

Figure 5. Effect of green tea polyphenols (0.075%) on glucose uptake by rat adipocytes. Values are shown as the mean ± SD ($n = 4$). *, $P < 0.05$ compared to the control group.

The plasma TG and FFA levels in the green tea group decreased significantly after 12 weeks of supplementation. Serum TG and FFA levels are significantly lower in rats given green tea for 6 weeks (*9*), and hamsters given green tea extract or green tea epicatechins have lower serum TG and cholesterol levels in a high-fat diet model (*34*, *35*). This effect, due to inhibition of cholesterol or fatty acid synthesis, is most probably mediated by an effect on the absorption of dietary fat and cholesterol (*35*). In addition, green tea and tea polyphenols can inhibit the activity of acetyl-Co A carboxylase, thus inhibiting TG synthesis (*36*).

Little has been reported on the relationship between tea or tea polyphenols and diabetes mellitus (*16−18*). Green tea and black tea extracts have been shown to have a hypoglycemic effect in STZ-induced diabetic rats (*18*). Deng et al. (*33*) found that serum glucose and TG levels in rats were decreased after green tea supplementation. In our study, an OGTT was performed, and the results showed that plasma insulin levels in the green tea group were lower than those in the control group after 12 weeks of supplementation. This shows that the insulin sensitivity of the rats was increased as a result of green tea supplementation. The adipocyte glucose uptake and insulin-binding results were consistent with the OGTT results. The green tea group had higher glucose uptake activity than the control group, showing that green tea supplementation resulted in higher insulin sensitivity. The insulin competitive binding results provided further evidence for the beneficial effect of green tea, which led to an increase in the number of high-affinity insulin receptor binding sites. Thus, compared to the control group, the green tea group showed increased insulin binding, which helped adipocytes to increase their glucose uptake, that is, it is beneficial in increasing insulin sensitivity.

Oxidative stress is suggested to be involved in the onset and progression of diabetes (*38*). Reactive oxygen species have been reported to interfere with insulin signaling at various levels and are able to inhibit the translocation of glucose transporter IV in the plasma membrane (*38*). A protective effect of antioxidants on diabetes was also reported (*39*, *40*). The antioxidant activity of green tea is well-known (*7*, *8*), and the benefits associated with green tea for atherosclerosis, hypertension, tumor, and immune response are generally attributed to the antioxidant activity of green tea. In our study, green tea was found to

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increase insulin sensitivity; in addition, Gomes et al. (*18*) also found that green tea had a hypoglycemic effect in STZ-induced diabetic rats, which are known to have high oxidative stress (*41*). We therefore suggest that the antioxidative effect of green tea might be one of the possible reasons for its ability to increase insulin sensitivity.

With regard to active components, Kao et al. (*42*) repeatedly injected SD rats by intraperitoneal injection with epigallocatechin gallate and found that levels of endocrine factors, such as insulin, glucose, TG, and cholesterol, were lowered; EGCG is therefore regarded as having a modulating effect on the endocrine system and might have improving effects on obesity, diabetes, and cardiovascular disease. Green or black tea stimulated adipocyte glucose uptake by treating rat epididymal fat pad adipocytes with aqueous extracts of medicinal plants, which indicated that they have an insulin-like effect (*43*). When poly(vinylpyrrolidone) was added to the insulin-like medicinal plant aqueous extracts to remove polyphenols, the activity was lost, which suggested that the insulin-like effect might be due to polyphenols. Anderson and Polansky (*23*) also have proved that green tea can enhance in vitro insulin activity, and the primary active component in green tea was shown to be epigallocatechin gallate. In the present study, we showed green tea polyphenols increased glucose uptake of adipocytes, which was consistent with the previous studies, and suggested that polyphenols in green tea might be responsible for increasing insulin activity.

In conclusion, green tea supplementation in the form of regular tea infusion could increase insulin sensitivity in rats by increasing the glucose uptake and insulin binding of adipocytes. Green tea polyphenols increased in vitro glucose uptake by adipocytes, suggesting that it might be one of the active components. Animal studies of the green tea polyphenols are needed to confirm this point.

ACKNOWLEDGMENT

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