Original Contribution

Green tea catechins ameliorate adipose insulin resistance by improving oxidative stress

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Abstract

Epidemiological data have suggested that drinking green tea is negatively associated with diabetes, and adipose oxidative stress may have a central role in causing insulin resistance, according to recent findings. The aim of this work is to elucidate a new mechanism for green tea's anti-insulin resistance effect. We used obese KK-ay mice, high-fat diet-induced obese rats, and induced insulin resistant 3T3-L1 adipocytes as models. Insulin sensitivity and adipose reactive oxidative species (ROS) levels were detected in animals and adipocytes. The oxidative stress assay and glucose uptake ability assay were performed, and the effects of EGCG on insulin signals were detected. Green tea catechins (GTCs) significantly decreased glucose levels and increased glucose tolerance in animals. GTCs reduced ROS content in both models of animal and adipocytes. EGCG attenuated dexamethasone and TNF-α promoted ROS generation and increased glucose uptake ability. EGCG also decreased JNK phosphorylation and promoted GLUT-4 translocation. EGCG and GTCs could improve adipose insulin resistance, and exact this effect on their ROS scavenging functions.

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Introduction

The metabolic syndrome is characterized by a group of metabolic risk factors in an individual, including abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, and insulin resistance or glucose intolerance [1]. The dominant underlying risk factors for metabolic syndrome are abdominal obesity and insulin resistance [2]. Obesity is the causal component in this syndrome [3,4], but the mechanistic role of obesity has not been fully elucidated. The oxidative stress in adipocytes induced by fat overload might be the origin of the metabolic syndrome caused by obesity [5]. Oxidative stress in adipocytes impairs insulin signals and increased insulin-stimulating glucose uptake [6]. As adipocytes are not only a glucose and fat storage position, they also could secrete cytokines to affect glucose and lipid homeostasis [7,8]. The fat-overloaded adipocytes secreted ROS, TNF-α, resistin, and free fatty acids to cause insulin resistance in muscles and liver [5,9]. ROS secreted by adipocytes could change the whole body redox system through transportation of blood [5].

Drinking of green tea has been found to have an antidiabetes effect for a long time [10–12]. Kim et al. found that GTCs could protect pancreas from oxidative damage [13]. Recent findings show that GTCs could increase insulin sensitivity in diabetic animals [14,15]. GTCs could enhance GLUT-4 expression, increase glucose tolerance, and promote glucose uptake in adipocytes and muscles [16,17]. Also GTCs could decrease oxidative stress in diabetic rats [18–20]. But how GTCs improved the impaired insulin resistance is still not fully understood.

As GTCs are one of the most used antioxidants, and epidemiological evidence also shows that oxidative stress is associated with insulin resistance [12,21], and especially that oxidative stress in adipocytes might be the connection between obesity and type 2 diabetes (T2D), we propose that one mechanism of GTCs antibiabetic effect is that GTCs decrease oxidative stress in adipocytes and improve insulin sensitivity. GTCs could scavenge ROS in other tissue directly and ROS secreted by adipocytes also could act on other tissues; thus for the whole body, especially muscle and liver, oxidative stress is decreased.

Since oxidative stress in adipocytes plays a central role in obesity-caused insulin resistance and T2D, we conducted our research in obese and diabetic KK-ay mice, diet-induced obese rats, and 3T3-L1 adipocytes to detect the improvement of insulin signals and the
enhancement of glucose uptake by GTCs through amelioration of oxidative stress.

**Experimental procedures**

**Animals and cell culture**

Eight-week-old female obese KK-ay and C57BL/6j mice were purchased from Institute of Laboratory Animal Science, Chinese Academy of Medical Science, and housed in a SPF (specific parasite free) environment at 22 °C with a 12-h light–dark cycle. All mice were fed a high fat diet (15% saturated fat, 1% cholesterol, 84% chow diet) for 1 week to allow adaptation to the environment and diet. KK-ay mice are confirmed to exhibit obesity at 9 weeks of age. Mice were randomly divided into 4 groups, depending on the regimen of gavage: Vehicle-fed C57 (C57), Vehicle-fed KK-ay (KK), GTCs 150 mg/kg/day-fed (KK+Low), GTCs 300 mg/kg/day-fed (KK+High). Mice were treated with GTCs for 4 weeks. GTCs used in this work are 98% pure containing 50% EGCG ((-)-epigallocatechin gallate), 22% ECG ((-)-epicatechin gallate), 18% EGC ((-)-epigallocatechin), and 10% EC ((-)-epicatechin) (analyzed by HPLC). The food and water intakes by the animal and nutritional status of the animal groups were measured every day and compared.

All rats were subdivided into 4 groups: Vehicle-fed + chow diet group (Chow), GTCs 20 mg/kg/day-fed + chow diet group (Chow+GTCs), Vehicle-fed + high fat diet group (HF), GTCs 20 mg/kg/day-fed high fat diet group (HF+GTCs). Rats were treated with GTCs for 45 days.

3T3-L1 preadipocytes were cultured and differentiation was induced as described previously [22]. The solution of EGCG was freshly prepared in potassium phosphate buffer (PBS), pH 7.4. EGCG (Sigma, St Louis, MO, USA) was added to medium after cells had fully differentiated.

**RT-PCR**

The total RNA was extracted from adipose of abdomen or 3T3-L1 cells with TRIzol Regent (Invitrogen, Carlsbad, CA, USA). The total RNA (2 μg) was reversely transcribed using the SuperScript First-Strand Synthesis System (Invitrogen). The primers were designed according to the sequences of GenBank. A standard (25 μl) of polymerase chain reaction containing 2 μl of the reverse transcriptase was performed with TaKaRa EX Taq Hot Start Version (Takara Japan) in MJ Research PTC-200 Peltier Thermor Cycler. The PCR products (10 μl) were separated on 2% agarose gels by electrophoresis.

**Western blot**

Adipose tissues homogenates and cell lysates were centrifuged at 12,000 g for 5 min at 4 °C and the supernatants were collected. Standard protocols were used for Western blot. The band intensities were quantified using an image analyzing software (NIH Image). Antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

**Glucose uptake assay**

3T3-L1 Cells (5×10⁵ /well) plated in a 12-well plate in serum containing medium (DMEM) overnight were washed twice in PBS, incubated in serum-free medium for 2 h, and then incubated in

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**Fig. 1.** GTCs feeding decreased the blood glucose content of KK-ay mice. GTCs feeding decreased random blood glucose content (RBG) (A), fasting blood glucose content (FBG) (B), and 2-h blood glucose content (2HGB) (C). A The standards of normal RBG, FBG, and 2HGB are 11, 7, and 11 mM, respectively. (D) GTCs feeding increased glucose tolerance of KK-ay mice. Values are mean±SE of 10 animals per group for each measurement. * indicates a significant difference between GTCs feeding and vehicle feeding groups (P<0.05).
1 ml/well PBS containing 200 nM insulin for 30 min at 37 °C. After one wash in PBS, the cells were incubated in 1 ml PBS containing 0.1 mM 2-deoxyglucose and 1 mCi/ml 2-deoxy-D-[3H]glucose for 5 min, washed three times in ice-cold PBS, and then solubilized in 0.4 ml of 1% SDS. [3H]Glucose uptake was detected in 4 ml of scintil-late using a Beckman LS6500 scintillation counter. Nonspecific

Fig. 2. GTCs feeding ameliorated oxidative stress in KK-ay mice. (A) GTCs feeding decreased ROS content in serum, (B) MDA content in serum, (C) blood. ROS was detected by the ERS spin trapping technique and MDA was detected by the TAB method, respectively, in the samples of mice after treatment by GTC for 45 days. The inset is the spin trapped ROS by PBN and measured by ESR. Details are described in the methods section. Values are mean ± SE of 10 animals per group for each measurement. * indicates a significant difference between GTCs feeding and vehicle feeding groups (P<0.05).

Fig. 3. Effects of GTC on phospho-JNK (P-JNK) and GLUT-4 level and in subcutaneous visceral white adipose tissues and content of GLUT-4 in plasma membrane protein of KK-ay mice. GTCs feeding reduced the phospho-JNK (P-JNK) (A,B) and GLUT-4 (A,C) level in subcutaneous and visceral white adipose tissues (sWAT and vWAT). GTCs feeding reduced the content of GLUT-4 (A,D) in both total cellular protein and plasma membrane protein. The column graphs are the statistical results of band intensities. Values are mean ± SE of 10 animals per group for each measurement. * indicates a significant difference between GTCs feeding and vehicle feeding groups (P<0.05).
deoxyglucose uptake is measured in the presence of 20 mM cytochalasin B and is subtracted from the total uptake to obtain specific glucose uptake. Insulin-stimulated glucose uptake was assayed with 2-deoxyglucose and 2-deoxy-D-[3H]glucose, and glucose uptake assay was performed after the cells were treated by EGCG and DEX or EGCG and TNF-α.

**Measurement of intracellular ROS in cell**

Production of ROS including mainly hydrogen peroxide but also other ROS [22] was monitored spectrophotometrically using dichlorofluorescein (DCF)-DA. Oxidation of DCF-DA by peroxides yielded the fluorescent derivative DCF. Two micromoles of DC-DA (final concentration) in N,N-dimethylformamide was incubated with cells for 50 min. Loaded cells were washed three times, and the fluorescence intensity of DCF was determined using a CytoFlour 4000 fluorescence spectrophotometer with the excitation wavelength at 485 nM.

**ROS detection with NBT in 3T3-L1 adipocytes**

3T3-L1 preadipocytes were grown to confluence and induced to differentiate into adipocytes, as described. ROS production was detected by nitroblue tetrazolium (NBT) assay. NBT is reduced by ROS to a dark-blue, insoluble form of NBT called formazan. At Days 0, 2, 4, and 8 after induction, 3T3-L1 cells were incubated for 90 min in PBS containing 0.2% NBT. Formazan was dissolved in 50% acetic acid, and the absorbance was determined at 560 nm.

**Detection of ROS with ESR**

ROS in blood and serum were detected according to a previously published method with minor modifications [22]. Briefly, 0.6 ml blood or 0.3 ml serum was mixed with 1 ml of a spin trap solution containing 100 mM N-tert-butyl-α-phenylnitrene (PBN) and 2 mM diethyldithiocarbamate (DETA), 10 mM HEPES in ice-cold phosphate-buffered saline, and vortexed for 30 s. Then 500 μl of ethyl acetate was added, vortexed for 30 s, and centrifuged at 10,000 g for 5 min. The ethyl acetate phase was transferred to a quartz cell for ESR study at room temperature. The height of the second peak from the ESR spectra of PBN spin adducts was measured. The Bruker ER-200 X-band ESR spectrometer settings for trapping oxygen radicals were as follows: center field, 3445 G; scan range, 200 G; microwave frequency, 9.47 GHz; modulation frequency, 100 kHz; microwave power, 20 mW; modulation amplitude, 1 G.

**Measurement of thiobarbituric acid reacted substances (TBARS)**

TBARS assay is well known to generate noninterpretable scientific data but it still can be used to indicate the oxidation damage. So the
level of lipid peroxidation was measured by determining TBARS [23]. Blood and serum were collected on ice and then mixed with 1 ml of 0.8% (w/v) trichloroacetic acid, 0.8 ml dd-water, respectively. After concussing, the samples were incubated for 60 min in boiling water, and TBARS were extracted in 3 ml of n-butanol. Following centrifugation at 4400 g for 10 min, the absorption of the butanol layer was measured at 532 nm. 1,1,3,3-Tetraethoxypropane (TMP) served as MDA standards. TBARS levels were expressed as the ratio of the value obtained from treated cultures and control cultures.

**Glucose tolerance test (GTT)**

After overnight fasting, glucose solution at a dose of 2 g/kg body weight was administered by intraperitoneal injection. The whole glucose levels were assessed at 0, 15, 30, 60, 90, and 120 min using a Super Glucocard II glucometer.

**Cell transfection and GLUT-4 translocation detection**

To image GLUT-4 translocation, fully differentiated 3T3-L1 adipocytes were transfected with the GLUT-4-eGFP plasmid using Lipofectamine 2000 and were allowed to express the fusion gene for 16–24 h. A 0.1 μM insulin was used to induce the translocation of GLUT-4-eGFP to plasma membrane. In experiments, 20 nM DEX, 20 nM DEX + 1 μM EGCG, 40 ng/ml TNF-α, or 40 ng/ml TNF-α + 1 μM EGCG was added to the medium 12 h before the addition of insulin. For living cell measurements, we used a laser-scanning confocal microscope (Olympus FV500, Tokyo, Japan) to image GLUT-4-eGFP translocation to plasma membrane. GLUT-4-eGFP was observed at 488 nm.

**Statistics**

The results are presented as means with standard errors. Statistical analysis was performed using the ORIGIN program. Differences between the groups were established using the least significant difference (LSD) test or ANOVA. Significance was assessed at the P<0.05 level.

**Results**

**GTCs ameliorate diabetic phenotype of KK-ay**

Serum glucose level is an important index for diabetes. GTCs feeding for 4 weeks decreased the random blood glucose (RBG) content by ≈30.4% (Low) and 51.2% (High) (Fig. 1A), the fasting blood glucose (FBG) content by ≈31.6% (Low) and 43.3% (High) (Fig. 1B), and the 2-h blood glucose (2HBG) content by ≈26.5% (Low) and 49.7% (High) (Fig. 1C), respectively. The standards of normal RBG, FBG, and 2HBG are 11, 7, and 11 mM, respectively. The mean FBG of the high GTCs group was 6.7 mM, which was lower than normal standard. The mean RBG and 2HBG of the high GTCs group were 16.2 and 13.5 mM, respectively, which were all near the normal standards.

**GTCs attenuate oxidative stress in KK-ay mice**

Adipose oxidative stress induced by fat accumulation caused oxidative stress in the whole body. Our results showed that ROS content in KK-ay blood serum was increased (Fig. 2A). Also ROS secreted by adipocytes increased serum and total blood MDA contents (Figs. 2B and C). GTCs feeding decreased the ROS content of serum (Fig. 2A).
and decreased the MDA content in serum and blood of KK-ay mice (Figs. 2B and C). These data suggested GTCs feeding reduced adipose ROS content and adipose ROS secretion.

**GTCs suppressed oxidative-related signal and increased GLUT-4 expression in KK-ay mice**

Extraordinary phosphorylation of JNK in adipocytes and muscles induced insulin resistance [24,25], and oxidative stress suppressed GLUT-4 expression and translocation to plasma membrane by activating JNK. GTCs feeding reduced JNK phosphorylation in adipose tissues (Fig. 3) and increased GLUT-4 expression as a result (Fig. 3). GTCs feeding also increased GLUT-4 content in plasma membrane which meant that GLUT-4 translocation had been enhanced (Fig. 3). These data suggested that GTCs increased insulin sensitivity by suppressing the JNK pathway.

**GTCs improved diet-induced obese (DIO) rat glucose metabolism**

In DIO rats, serum glucose content was increased compared with chow diet control (Fig. 4A). High fat food also caused an increase of ROS content in rat blood serum (Fig. 4B). GTCs feeding decreased glucose absorption and decreased ROS content in diet-induced obese animals.

**EGCG recovered the impaired insulin-stimulated glucose uptake of 3T3-L1 adipocytes**

DEX and TNF-α caused insulin resistance by inducing oxidative stress in adipocytes [8]. Apocynin is an inhibitor of NADPH-oxidase and could reduce intracellular ROS production. NADPH-oxidase was widely considered to be the major source of oxidative stress caused by fat accumulation. DEX or TNF-α incubation reduced insulin-stimulated 2-deoxy-D-[3H]glucose uptake (Fig. 5). As a positive control, apocynin prevented 2-deoxy-D-[3H]glucose uptake from DEX or TNF-α impairment. EGCG treatment attenuated the effect of DEX or TNF-α and increased the impaired glucose uptake. We also measured the effect of EGCG on the glucose uptake by the cells. It was found that only the concentration of 5 μM had significantly increased the glucose uptake compared with that of the untreated group. However, EGCG treatment still attenuated the effect of DEX or TNF-α and increased the impaired glucose uptake and this factor was considered. These data suggested that EGCG could protect adipocyte glucose uptake from oxidative stress disturbance.

**EGCG reduced DEX or TNF-α-induced ultra ROS generation**

NBT is reduced by ROS to a dark-blue, insoluble form of NBT called formazan. Formazan production was increased by DEX or TNF-α, and EGCG reduced formazan production (Fig. 6A). Formazan could be dissolved in 50% acetic acid, and the absorbance was determined at 560 nm. The absorbance of adipocyte homogenates after NBT dying was increased by DEX or TNF-α, but decreased by EGCG (Figs. 6B and C). Detection of ROS content with the redox-sensitive dye DCFH-DA also showed that DEX or TNF-α increased ROS contents in adipocytes and EGCG attenuates these increases (Figs. 6D and E). We also measured the effect of EGCG on ROS in the cells. It was found that there was no significant change of ROS in the used concentration compared with that of the untreated group. These data suggested that EGCG ameliorated oxidative stress induced by DEX or TNF-α.

**EGCG reduced JNK phosphorylation in adipocytes**

DEX or TNF-α caused an increased level of phosphor-JNK in adipocytes (Fig. 7). When the DEX or TNF-α-treated adipocytes were incubated with EGCG, increases of JNK phosphorylation were attenuated.

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**Fig. 7.** Effect of EGCG on the phosphorylation level of JNK in 3T3-L1 adipocytes. Phosphorylation level of JNK in 3T3-L1 adipocytes incubated with EGCG and DEX (A,A1) or with EGCG and TNF-α (B,B1) was detected by Western blot at Day 16 after the cells were treated by EGCG and DEX or EGCG and TNF-α. Values are mean ± SE. * indicates a significant difference between EGCG groups and DEX or TNF-α group (P<0.05), † indicates a significant difference between EGCG groups and control group (P<0.05), and & indicates a significant difference between DEX or TNF-α groups and control group considering the effects of EGCG on the phosphorylation level of JNK in 3T3-L1 adipocytes (P<0.05).
We also measured the effect of EGCG on JNK phosphorylation in the cells. There was no significant change of JNK phosphorylation in the used concentration compared with that of the untreated group. These data suggested that DEX or TNF-α activated the JNK pathway, but EGCG suppressed this activation.

EGCG enhanced translocation of GLUT-4 to plasma membrane

GLUT-4 translocation using GLUT-4-eGFP chimeric protein and dynamics of its intracellular distribution after addition of stimulants were detected with confocal microscopy (Fig. 8). Treatment of adipocytes with DEX or TNF-α alone attenuated the fluorescence intensity of GLUT-4-eGFP in plasma membrane after insulin stimulation. But EGCG elevated the translocation of confusion GLUT-4-eGFP to plasma membrane.

EGCG altered synthesis of adipokines

Adiponectin and resistin were synthesized in adipocytes. The effects of DEX and EGCG on mRNA levels of adiponectin and resistin were measured (Fig. 9). DEX increased the mRNA level of resistin but decreased the mRNA level of adiponectin. EGCG reduced the effects of DEX on adipocytes: decreased adiponectin gene expression and increased resistin gene expression. We also measured the effect of EGCG on the mRNA level of adiponectin and resistin in the cells. It was found that only the concentration of 5 μM had significantly increased and decreased adiponectin and resistin, respectively, compared with those of the untreated group. However, EGCG treatment still significantly decreased the effect of DEX on adiponectin and resistin and this factor was considered.

Discussion

Drugs that reverse insulin resistance are of importance as insulin resistance is one of the most important characters of metabolic syndrome and frequently associated with type 2 diabetes [26,27]. It was well established that GTCs administration improved metabolic syndrome and type 2 diabetes [10,12,28]. In this study, the effects of GTC on type 2 diabetes were studied and the mechanism of GTCs on the insulin sensitive effect was investigated by feeding of GTCs to obese KK-ay mice, DIO obese rats, and 3T3-L1 adipocytes, all ameliorated insulin resistance to varying degrees.

KK-ay mice are genetically predisposed to obesity and type 2 diabetes, accompanied with a high blood glucose level and glucose intolerance. GTCs feeding improved RBC, FBG, and 2HBB. For the glucose tolerance assay, GTCs feeding delayed the appearance of a glucose peak value and decreased the glucose level rapidly, compared with those of the control group. Thus GTCs feeding significantly increased the glucose uptake ability of KK-ay and enhanced insulin sensitivity. In DIO obese rats, similar results were also obtained.

As liver, muscles, and adipose tissues store most of the glucose after eating and ROS in adipocytes has been reported as the common trigger for different types of insulin resistance [6], we detected ROS levels in animal blood serum. GTCs feeding significantly decreased ROS levels in blood serum. ROS produced by adipose tissues not only influence adipose redox, but also result in oxidative stress in other tissues, such as liver and muscles [5,29]. Our results showed that GTCs feeding ameliorated blood oxidative stress. Epidemiological investigation results showed that oxidative stress in many tissues was proven to be consistent with insulin resistance, and pathological research also found an important role for oxidative stress in insulin resistance [30]. Decreased ROS levels in blood and other tissues might increase insulin sensitivity, and may be the mechanism of GTCs’s insulin sensitizing effects. The blood TBARS and ESR spin trap analysis cannot reveal exactly which were oxidized and what free radicals were generated; however, they were used to compare the effect of GTC on ROS generation and oxidative stress.

Oxidative stress in adipocytes plays a central role in whole-body insulin resistance and ROS secreted by adipocytes leads to insulin resistance in many tissues. We detected the effect of GTCs on insulin signals in 3T3-L1 adipocytes. To detect GTCs’ effect in different types of insulin resistance, we induced insulin resistance in mature 3T3-L1 adipocytes by treatment with the cytokine TNF-α and the other with the glucocorticoid DEX. TNF-α-induced oxidative stress and insulin resistance in adipocytes through its membrane receptor while DEX through inflammatory reactions [6]. In KK-ay adipose tissues, NADPH oxidase was proved as the major source of oxidative stress, and an inhibitor of NADPH oxidase, apocynin, was used to treat adipocytes in this experiment as a positive control [5]. After being stimulated by insulin, adipocytes had a rapid increase in glucose uptake ability. Our results showed that apocynin partially ameliorated the impaired glucose-uptake ability by TNF-α and DEX (Fig. 5). Thus both TNF-α and DEX-induced insulin resistance relied on a ROS production process. EGCG increased the insulin-stimulated glucose uptake ability of adipocytes. As a potent antioxidant, EGCG may ameliorate TNF-α and DEX-induced insulin resistance by scavenging ROS.

Glucocorticoid dexamethasone has different effects on the generation of ROS through different pathways in different cell types. Dexamethasone is a suppressant of the oxygen burst phenomenon in
with insulin resistance [37,38]. EGCG treatment decreased DEX and narily high levels of phosphor-JNK were found in the bodies of people the downstream reactions. Clinical research showed that extraordin-

dition [35,36]. Phosphor-JNK interfere with insulin signals and weaken cytes induced insulin resistance partly by activating JNK phosphoryla-

dation [35,36]. Phosphor-JNK interfere with insulin signals and delay GLUT-4 translocation to plasma membrane [39]. GLUT-4 is the major adipose

glucose inner transporter after stimulation by insulin [40]. EGCG de-

creased DEX and TNF-α-induced JNK phosphorylation and enhanced GLUT-4 translocation. EGCG increased the glucose uptake ability of adipocytes. This explained why EGCG promoted adipocytes to uptake more 2-deoxy-D-[3H]glucose than DEX or TNF-α impairing groups, when added to medium together with DEX or TNF-α.

Another pathway where oxidative stress in adipose tissue could induce whole-body insulin resistance is secreting cytokines. Based on their effects on lipids and glucose metabolism, adipokines were generally divided into two groups: the positive group, including leptin, adiponectin, etc.; and the negative group, including TNF-α, resis-
tin, etc. [7,8]. Adiponectin could stimulate adipose, muscles, and other tissues to uptake glucose while resistin could induce inflamm-
atory reactions in many glucose metabolism-related tissues [41,42]. Obesity-induced oxidative stress in adipocytes reduced adiponectin but increased resistin secretion in adipocytes, which in turn resulted in adipose, liver, and muscles insulin resistance. In our experiments, DEX and TNF-α increased ROS production in adipocytes, and thus reduced adiponectin synthesis and increased resistin synthesis. EGCG could weaken these changes caused by DEX and TNF-α as the results of its ROS scavenging effect.

There are several literature references showing that green tea cate-

echins have a direct inhibitory effect on glucose transport via GLUT-1. They investigated the effects of EC, EGC, Cg, and EGCg on the translo-
cation of GLUT-4 to the plasma membrane in 3T3-L1 cells. In the ab-
cence of insulin, EC and EGC at 50 μM significantly increased the amount of GLUT-4 in the plasma membrane fraction compared with DMSO as the vehicle control. On the other hand, in the presence of

macrophages and neutrophils through NADPH oxidase reported in the literatures [31,32]. However, also reported in the literature is that dexamethasone increased reactive oxygen species (ROS) levels in 3T3-L1 adipocytes. Using gene analysis and measuring the oxidation of the redox-sensitive dye DCF, Houists et al. studied insulin resistance resulting from the treatment of 3T3-L1 adipocytes with either the inflammatory cytokine tumor-necrosis factor-α (mouse TNF) or the glucocorticoid dexamethasone and they found that after treatment with the cytokine tumor-necrosis factor-α and the other with the glucocorticoid dexamethasone ROS levels are increased through insulin resistance and a nuclear hormone receptor in cell and animal models [33,34].

We detected ROS in adipocytes after treatment in turn. Both NBT dye and DCF-DA detection showed that TNF-α and DEX increased ROS levels in adipocytes, and EGCG treatment suppressed this effect. NBT was incubated with cells for 3 h while DCF-DA was incubated with the cells for 30 min. As NBT was reduced by ROS to formazan constantly, DCF-DA results reflected the final levels of ROS in adipocytes while NBT results reflected the accumulative effect of treatment of DEX or TNF-α with or without EGCG [5]. Overloaded ROS in adipocytes induced insulin resistance partly by activating JNK phosphorylation [35,36]. Phosphor-JNK interfere with insulin signals and weaken the downstream reactions. Clinical research showed that extraordin-
arily high levels of phosphor-JNK were found in the bodies of people with insulin resistance [37,38]. EGCG treatment decreased DEX and TNF-α-induced JNK phosphorylation by scavenging ROS. Phosphor-JNK interfere with insulin signals and delay GLUT-4 translocation to plasma membrane [39]. GLUT-4 is the major adipose

![Fig. 9. Effects of EGCG on expression of adiponectin and resistin in adipocytes. Expression of adiponectin and resistin in 3T3-L1 adipocytes incubated with EGCG and DEX and detected by RT-PCR at Day 16 after the cells were treated by EGCG and DEX. Values are mean±SE. * indicates a significant difference between EGCG groups and DEX group (P<0.05), # indicates a significant difference between DEX groups and control group (P<0.05), and & indicates a significant difference between DEX groups and control group considering the effects of EGCG on expression of adiponectin and resistin in 3T3-L1 adipocytes (P<0.05).](image-url)
100 nM insulin, Cg and ECGc at 50 μM significantly decreased the amount of GLUT-4 in the plasma membrane fraction compared with insulin as the positive control. For GLUT-1, the amount of it on the plasma membrane fraction remained unchanged. Moreover, these catechins did not affect the expression levels of GLUT-4 in the cells. Thus, nongallate-type catechins promoted the translocation of GLUT-4, whereas gallate-type catechins decreased the insulin-induced translocation. [43]. We studied the effect of GLUT-4 translocation using GLUT-4-eGFP chimeric protein and the dynamics of its intracellular distribution after addition of stimulants were detected with confocal microscopy (Fig. 8). We found that treatment of adipocytes with DEX or TNF-α alone attenuated the fluorescence intensity of GLUT-4-eGFP in plasma membranes after insulin stimulation. But 1 μM ECGG elevated the translocation of confusion GLUT-4-eGFP to plasma membrane. We used EGCG at 1 μM in the experiment, which is similar to the physiological concentration and is 50 times lower than that used in the literature.

Obesity could induce insulin resistance for oxidative stress in adipocytes, and ROS play important roles in various types of insulin resistance. Based on our in vivo and in vitro results, we found that EGCG and GTCs could improve adipocyte insulin resistance, and exert this effect on their ROS scavenging functions. Although there is still much debate on the clinical usage of GTCs on type 2 diabetes, our results have found and explained the antiadipose insulin resistance effects of GTCs.

The nutrition status intakes by the animals were detected every day in our experiments and it was found that the food and water intakes by the animals were not significantly different and they were comparable to the concentrations used in the literature [44]. The GTCs used in the experiments in the reports were higher and more rapid than the green tea intake in the human epidemiological studies, yet, the food intakes by the animals were not significantly changed [44]. But if the concentration is higher, GTCs react with food in the gastrointestinal, disturb the digestion and absorption of lipid, and reduce energy intake. Lee et al. and other reported that [45–46] the peak concentrations of ECGG, EGCG, EC in the plasma were about 1–5 μM and it can be maintained for about 3 h if a healthy person drank 20 mg/kg body weight. The concentrations of EGCG used in our 3T3-L1 adipocyte experiments were 1–5 μM which were comparable with the plasma concentrations.

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