Epigallocatechin Gallate Supplementation Alleviates Diabetes in Rodents¹

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Abstract

As the prevalence of type 2 diabetes mellitus is increasing at an alarming rate, effective nutritional and exercise strategies for the prevention of this disease are required. Specific dietary components with antidiabetic efficacy could be one aspect of these strategies. This study investigated the antidiabetic effects of the most abundant green tea catechin, epigallocatechin gallate (EGCG, TEAVIGO), in rodent models of type 2 diabetes mellitus and H4IIE rat hepatoma cells. We assessed glucose and insulin tolerance in db/db mice and ZDF rats after they ingested EGCG. Using gene microarray and real-time quantitative RT-PCR we investigated the effect of EGCG on gene expression in H4IIE rat hepatoma cells as well as in liver and adipose tissue of db/db mice. EGCG improved oral glucose tolerance and blood glucose in food-deprived rats in a dose-dependent manner. Plasma concentrations of triacylglycerol were reduced and glucose-stimulated insulin secretion was enhanced. In H4IIE cells, EGCG downregulated genes involved in gluconeogenesis and the synthesis of fatty acids, triacylglycerol, and cholesterol. EGCG decreased the mRNA expression of phosphoenolpyruvate carboxykinase in H4IIE cells as well as in liver and adipose tissue of db/db mice. Glucokinase mRNA expression was upregulated in the liver of db/db mice in a dose-dependent manner. This study shows that EGCG beneficially modifies glucose and lipid metabolism in H4IIE cells and markedly enhances glucose tolerance in diabetic rodents. Dietary supplementation with EGCG could potentially contribute to nutritional strategies for the prevention and treatment of type 2 diabetes mellitus. J. Nutr. 136: 2512–2518, 2006.

Introduction

In Asia, green tea is a widely consumed beverage and, for centuries, has been regarded to possess considerable health-promoting effects (1). In recent years, research has mainly focused on the effects of green tea in relation to the prevention of cancer (2) and cardiovascular disease (3). Furthermore, the anti-inflammatory (4), antiarthritic (5), antibacterial (6), antiangiogenic (7), antioxidative (8), antiviral (9), neuroprotective (10) and cholesterol-lowering effects (11) of green tea and isolated green tea constituents are under investigation.

The health-promoting effects of green tea are mainly attributed to its polyphenol content. Green tea is a rich source of polyphenols, especially of flavanols and flavonols, which represent ≈30% of fresh leaf dry weight (1). Catechins are the predominant form of the flavanols and are mainly composed of epigallocatechin gallate (EGCG)³, epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) (12). Recently, many of the aforementioned beneficial effects of green tea were attributed to its most abundant catechin, EGCG (13–15). It was previously shown that EGCG exerts potent antiobesity effects in mouse and rat models of diet-induced obesity, which are at least partly mediated via a direct impact of EGCG on adipose tissue (16,17). EGCG causes a dose-dependent decrease of in vitro adipocyte differentiation and downregulates the mRNA expression of several lipogenic genes in adipose tissue.

The prevalence of type 2 diabetes mellitus (T2DM) is estimated to increase dramatically during the next few years, reaching 300 million by 2025 (18). The increasing prevalence of diabetes is largely due to the rapid spread of obesity, which is considered the most important risk factor for T2DM (19). T2DM is characterized by an imbalance between insulin secretion of the pancreatic beta cells and insulin action on skeletal muscle, adipose tissue, and liver (20). It is generally accepted that loss of the early insulin secretory response initially leads to postprandial hyperglycemia (21). As the disease progresses, the increase in endogenous glucose production (EGP) by the liver contributes to the development of clinical fasting hyperglycemia (22). Therefore, enhancing early insulin secretion and reducing endogenous glucose production have become important goals for the treatment of T2DM.

A study by Waltner-Law et al. (23) provided compelling in vitro evidence that EGCG decreases glucose production of H4IIE rat hepatoma cells. The investigators showed, furthermore, that EGCG mimics insulin, increases tyrosine phosphorylation of the

¹ Supplemental Materials and Methods are available with the online posting of this paper at jn.nutrition.org.
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³ Abbreviations used: EGCG, epigallocatechin gallate; EGP, endogenous glucose production; GK, glucokinase; PEPCK, phosphoenolpyruvate carboxykinase; rosi, rosiglitazone; T2DM, type 2 diabetes mellitus.
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insulin receptor and the insulin receptor substrate, and reduces
gene expression of the gluconeogenic enzyme phosphoenolpyruvate
carboxykinase (PEPCK). If these effects are relevant for
the in vivo situation, then EGCG possesses the potential to exert
strong antidiabetic effects. Recently, green tea and green tea
extracts were demonstrated to beneficially modify glucose
metabolism in experimental models of T2DM (24,25). On the
other hand, there is only one in vivo study suggesting a glucose-
lowering effect of EGCG (26). In that study, EGCG was injected
into lean and obese Zucker rats. This route of administration
ensured suprapharmacologic plasma concentrations of EGCG
and resulted in markedly decreased blood glucose and insulin
levels. However, it is unclear whether these observations were
due to a direct glucose lowering effect of EGCG or an anorectic
effect caused by suprapharmacologic plasma concentrations of
EGCG.

It was shown that EGCG ameliorates cytokine-induced beta-
cell damage in vitro (27) and prevents the decrease of islet mass
induced by treatment with multiple low doses of streptozotocin
in vivo (28). However, in the latter study, streptozotocin was
coinjected with EGCG, which possesses strong antioxidative
activity (29). It is unclear whether the protective effects observed
in this study were due to direct inactivation of the injected
streptozotocin.

Thus, the antidiabetic effects of EGCG are still not entirely
clarified. The in vivo relevance of potentially antidiabetic green
tea catechins remains to be demonstrated. Therefore, we con-
donducted an in vivo study to explore the antidiabetic effects of
dietary supplementation with the most abundant green tea
catechin, EGCG. Additionally, we assessed the effects of EGCG
on the expression of genes involved in lipid and glucose me-
tabolism in H4IIE rat hepatoma cells as well as in liver and
adipose tissue of db/db mice.

Materials and Methods

Animal care. The present study was performed with permission of the
“Kantonele Veterinâramt Basel” according to Swiss law. The investiga-
tion conformed to the National Institutes of Health (NIH) Guide for the
Care and Use of Laboratory Animals (30). Mice and rats were
individually housed in standard macronol type 3 and 4 cages, respec-
tively. All animals were maintained on a 12-h light (300 Lux) and 12-h
dark cycle at a humidity of 55–60% and a temperature of 23 ± 1°C. All
animals consumed modified AIN-93 diets (Provimi Kliba AG) (31) and
water ad libitum.

Diabetes alleviation in db/db mice. The effect of dietary EGCG
(TEAVIGO, DSM Nutritional Products) supplementation on T2DM was
investigated by utilizing the db/db mouse model (BKS.Cg-m+/+ Lepd-db).
TEAVIGO is a highly purified extract from green tea leaves (Camellia
sinensis) containing >94% EGCG, <5% other catechins (<3% epi catechin gallate). Male db/db mice were purchased from Jackson
Laboratories at an age of 5 wk. After an acclimation period of 2 wk, mice
consumed a modified AIN-93 diet containing EGCG at concentrations
of 2.5, 5.0, or 10.0 g/kg of diet (EGCG 0.25%, 0.5%, or 1% w:w, n = 9/
group) or placebo (control, n = 9) for 7 wk. Five mice receiving the
thiazolidinedione rosiglitazone (Avandia, GlaxoSmithKline) at a con-
centration of 72.0 mg/kg of diet (Rosi 0.0072%) were used as a positive
control group. Food intake and body weight were determined weekly.
Blood glucose levels in fed mice were measured weekly (Glucotrend,
Roche Diagnostics). After 5 wk of dietary EGCG supplementation,
an oral glucose tolerance test was performed. Before application of an oral
glucose load (1 g/kg, Sigma), blood glucose levels were determined
in food-deprived mice. Blood glucose levels were measured 15, 30, 45, 60, 90, 120, 150, and 180 min after glucose application (Glucotrend, Roche
Diagnostics). After 6 wk of dietary EGCG supplementation, a modified
intraportal insulin tolerance test was performed. Immediately prior
to the test, all mice were deprived of food. Short-acting insulin (1 IU/kg,
Actrapid, Novo Nordisk Pharma GmbH) was administered and blood
glucose levels were measured 30, 60, 90, 120, 180, 240, 300, and 360
min after administration. At the end of the study (7 wk), blood samples
were collected in the morning from fed mice and analyzed for glucose,
free fatty acids, and triacylglycerol concentrations (Hitachi 912 Au-
tomatic Analyser). Plasma insulin was determined by use of an enzyme
immunometric assay kit (Merodia AB) in fed mice.

To determine the efficacy of low-dosage treatment, EGCG was ad-
ministered orally by gavage at dosages of 30 mg • kg⁻¹ • d⁻¹ (EGCG 30,
n = 10) and 100 mg • kg⁻¹ • d⁻¹ (EGCG 100, n = 10) for 2 wk in a
separate set of mice (10 wk of age). EGCG was dissolved in distilled
water and a total volume of 0.1 mL was administered daily. The control

Figure 1  Blood glucose concentrations during an oral glucose tolerance test (oGTT) (A, D), calculated area under the curve (AUC) of the oGTT (B, E) and blood glucose concentrations of food-deprived db/db mice (C, F). Mice consumed diets supplemented with 0.25%, 0.5%, or 1% EGCG (n = 9) or 0.0072% rosiglitazone (n = 5) for 5 wk (A, B, C), or were administered orally by gavage 30 or 100 mg • kg⁻¹ • d⁻¹ EGCG (n = 10) or 10 mg • kg⁻¹ • d⁻¹ rosiglitazone (n = 5) for 2 wk (D, E, F). Values are means ± SEM. Means without a common letter differ, P < 0.05.
TABLE 1  Plasma concentrations of glucose, insulin, free fatty acids, and triacylglycerol in fed db/db mice and ZDF rats after supplementation with EGCG or rosiglitazone

<table>
<thead>
<tr>
<th></th>
<th>Glucose (mmol/L)</th>
<th>Insulin (nmol/L)</th>
<th>Free fatty acids (mmol/L)</th>
<th>Triacylglycerol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse feeding study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>53.7 ± 2.2</td>
<td>0.14 ± 0.02</td>
<td>0.48 ± 0.04</td>
<td>3.8 ± 0.6</td>
</tr>
<tr>
<td>EGCG, 0.25%</td>
<td>52.4 ± 2.3</td>
<td>0.30 ± 0.10</td>
<td>0.44 ± 0.03</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>EGCG, 0.5%</td>
<td>42.1 ± 2.4</td>
<td>0.51 ± 0.09</td>
<td>0.42 ± 0.03</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>EGCG, 1%</td>
<td>33.9 ± 2.5</td>
<td>0.96 ± 0.24</td>
<td>0.41 ± 0.04</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Rosi, 0.0072%</td>
<td>14.7 ± 1.5</td>
<td>0.97 ± 0.20</td>
<td>0.16 ± 0.04</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td><strong>Mouse gavage study</strong></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>53.3 ± 2.5</td>
<td>0.14 ± 0.03</td>
<td>0.66 ± 0.08</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>EGCG 30</td>
<td>49.4 ± 2.3</td>
<td>0.26 ± 0.04</td>
<td>0.62 ± 0.03</td>
<td>2.4 ± 0.2</td>
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<tr>
<td>EGCG 100</td>
<td>47.7 ± 1.9</td>
<td>0.38 ± 0.09</td>
<td>0.61 ± 0.02</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>Rosi 10</td>
<td>27.2 ± 3.3</td>
<td>0.28 ± 0.04</td>
<td>0.32 ± 0.05</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td><strong>Rat feeding study</strong></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>36.5 ± 1.0</td>
<td>0.40 ± 0.07</td>
<td>0.29 ± 0.02</td>
<td>7.9 ± 0.7</td>
</tr>
<tr>
<td>EGCG, 0.5%</td>
<td>30.7 ± 1.9</td>
<td>0.37 ± 0.08</td>
<td>0.18 ± 0.03</td>
<td>6.4 ± 1.2</td>
</tr>
<tr>
<td>Rosi, 0.0036%</td>
<td>9.9 ± 0.4</td>
<td>0.18 ± 0.03</td>
<td>0.19 ± 0.03</td>
<td>1.5 ± 0.2</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. For each study, means in a column with superscripts without a common letter differ, P < 0.05.
2 db/db mice were supplemented with placebo or 0.25%, 0.5%, or 1% w:w EGCG, (n = 9), or 0.0072% rosiglitazone (n = 5) for 7 wk.
3 db/db were administered orally by gavage with placebo or 30 or 100 mg · kg⁻¹ · d⁻¹ EGCG (n = 10) or 10 mg · kg⁻¹ · d⁻¹ rosiglitazone (n = 5) for 2 wk.
4 ZDF rats were fed a control diet or supplemented with 0.5% EGCG or 0.0036% rosiglitazone (n = 11) for 11 wk.

Results

**Diabetes alleviation in db/db mice.** Dietary EGCG supplementation for 5 wk improved oral glucose tolerance in db/db mice in a dose-dependent manner (Fig. 1A, B). When EGCG was applied orally by gavage for 2 wk, a dosage of 100 mg · kg⁻¹ · d⁻¹ improved oral glucose tolerance (Fig. 1D, E).

A pronounced decrease of glucose levels was observed in food-deprived db/db mice treated with EGCG (Fig. 1C, F). Dietary supplementation with 0.25%, 0.5%, and 1% EGCG resulted in reductions of glucose levels in food-deprived mice by 23.0%, 35.2%, and 47.6%, respectively. Compared with group (control, n = 10) was administered 0.1 mL distilled water. The thiazolidinedione rosiglitazone (Avandia, GlaxoSmithKline) was suspended in distilled water and administered at a dosage of 10 mg · kg⁻¹ · d⁻¹ in a positive control group (Rosi 10, n = 5). After 2 wk of application, an oral glucose tolerance test was carried out as described above. Plasma concentrations of insulin in fed mice were also determined.

**Diabetes alleviation in ZDF rats.** The effect of dietary EGCG supplementation on T2DM was further investigated in ZDF rats (ZDF/Gmi-Crl-Lepdb). Male ZDF rats were purchased from Charles River at an age of 5 wk. After an acclimation period of 2 wk, rats consumed a modified AIN-93 diet containing EGCG at a concentration of 5 g/kg of diet (EGCG 0.5% w:w, n = 10) or placebo (control, n = 10) for 10 wk. Ten rats receiving the thiazolidinedione rosiglitazone (Avandia, GlaxoSmithKline) at a concentration of 36.0 mg/kg of diet (Rosi 0.0036%) were used as a positive control group. Food intake and body weight were determined twice per wk. After 10 wk of treatment, an oral glucose tolerance test was carried out as described above. The plasma concentration of insulin was determined prior and 15, 30, and 45 min after glucose administration, as described above. At the end of the study, blood of all rats was collected, the plasma prepared and stored at −80°C until analysis of total EGCG plasma concentration was performed, as previously described (32).

**Real-time quantitative TaqMan RT-PCR.** Real-time quantitative TaqMan RT-PCR was used to quantify gene expression as previously described (16). Details are provided in the online supplemental material.

**Gene regulation of glucose and lipid metabolism in rat H4IE hepatoma cells.** The influence of EGCG on glucose and lipid metabolism-related genes were studied in rat hepatoma cell line H4IE cells (ATCC Global Bioresource Center). Cells were cultured and gluconeogenesis was induced as described by Walter-Law et al. (23). Cells were starved for 4 or 24 h before being treated with either insulin 10 nmol/L (positive control), or EGCG at 30 or 100 μmol/L. Final DMso concentration was standardized to 0.5%. Details are provided in the online supplemental material. Glucose-6-phosphotase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) expression levels were checked by real-time PCR as previously described (16). DNA microarray analysis was performed to investigate the effect of EGCG on genome-wide expression as previously described (33). Details are provided in the online supplemental material.

**Statistical analysis.** Data from db/db mice and ZDF rats are expressed as means ± SEM for animals in each group. Statistical significance of the mean differences between dietary groups was tested by 1-way ANOVA or repeated-measures ANOVA. Homogeneity of variance was tested using Levene’s test. When ANOVA revealed significant differences, means were compared using LSD post-hoc analysis (SPSS 13.0). Differences were considered significant at P < 0.05. RT-PCR data were analyzed by 1-way ANOVA. If significant differences were found, Dunnett’s test for multiple comparison (Statistica, version 5.3A, StatSoft) was used to compare each group to the control group. Significance was determined at P < 0.05. The gene chip data analysis was carried out using RACE-A (Roche Affymetrix Chip Experiment Analysis), a Roche proprietary software package for differential expression analysis, as described (33). Gene expression in EGCG treated cells was compared with that of solvent-treated cells. EGCG-induced changes in gene expression levels are reported as percentage of control relative to solvent-treated cells, which were set to 100%. Changes were considered significant at P < 0.05 for a single gene regulation study.

**Results**

**Diabetes alleviation in db/db mice.** Dietary EGCG supplementation for 5 wk improved oral glucose tolerance in db/db mice in a dose-dependent manner (Fig. 1A, B). When EGCG was applied orally by gavage for 2 wk, a dosage of 100 mg · kg⁻¹ · d⁻¹ improved oral glucose tolerance (Fig. 1D, E).

A pronounced decrease of glucose levels was observed in food-deprived db/db mice treated with EGCG (Fig. 1C, F). Dietary supplementation with 0.25%, 0.5%, and 1% EGCG resulted in reductions of glucose levels in food-deprived mice by 23.0%, 35.2%, and 47.6%, respectively. Compared with data from db/db mice and ZDF rats are expressed as percentage of control relative to solvent-treated cells, which were set to 100%. Changes were considered significant at P < 0.05 for a single gene regulation study.
control mice, dietary supplementation with 0.25%, 0.5%, and 1% EGCG decreased glucose levels in fed mice by 2.4%, 11.6%, and 36.9%, respectively (Table 1).

In mice that were deprived of food immediately before the test, the glucose-lowering effects of insulin were not significantly altered by EGCG treatment during the early time points of the insulin tolerance test. In control mice, blood glucose concentrations rebounded between 1 and 3 h after insulin administration to 69.9% of baseline. However, in mice treated with 1% EGCG, this rebound was not observed (Fig. 2A). The plasma concentrations of triacylglycerol were reduced by 0.25%, 0.5%, and 1% EGCG (Table 1). The plasma concentrations of insulin (plasma collected in the morning at 0800 from fed mice) were increased by EGCG supplementation in a dose-dependent manner (Table 1). In a previous study (16), we observed that EGCG supplementation tended to reduce plasma insulin concentrations in fed animals in 2 models of diet-induced obesity (C57BL/6J mice and Sprague Dawley rats) (for comparison, see Fig. 2B).

The cumulative food intake between control mice and mice supplemented with 0.25%, 0.5% or 1% EGCG did not differ (Table 2). However, the positive control rosiglitazone caused a significant reduction in cumulative food intake. Conversely, body weight increased in mice treated with rosiglitazone compared with control mice. EGCG supplementation did not exert a significant influence on final body weight. When EGCG was applied orally by gavage, cumulative food intake and body weight did not change compared with control mice (Table 2). Rosiglitazone applied orally by gavage for 2 wk increased body weight (Table 2).

**Diabetes alleviation in ZDF rats.** Dietary EGCG supplementation for 10 wk improved oral glucose tolerance in ZDF rats.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Cumulative food intake</th>
<th>Subcutaneous adipose tissue</th>
<th>Epididymal adipose tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse feeding study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34.4 ± 1.3a</td>
<td>270.8 ± 5.6b</td>
<td>2.9 ± 0.2b</td>
</tr>
<tr>
<td>EGCG, 0.25%</td>
<td>37.8 ± 0.9a</td>
<td>277.9 ± 5.9b</td>
<td>3.2 ± 0.2a</td>
</tr>
<tr>
<td>EGCG, 0.5%</td>
<td>37.4 ± 2.1b</td>
<td>282.7 ± 9.1b</td>
<td>3.3 ± 0.3b</td>
</tr>
<tr>
<td>EGCG, 1%</td>
<td>36.6 ± 0.8c</td>
<td>279.6 ± 5.9b</td>
<td>2.9 ± 0.1b</td>
</tr>
<tr>
<td>Rosi, 0.0072%</td>
<td>47.5 ± 1.5a</td>
<td>217.9 ± 6.0b</td>
<td>4.9 ± 0.1b</td>
</tr>
</tbody>
</table>

| **Mouse gavage study**  |                        |                             |                           |
| Control                  | 37.1 ± 0.8b            | 71.3 ± 3.0                  | ND                        |
| EGCG 30                 | 37.8 ± 0.8ab           | 71.1 ± 3.1                  | ND                        |
| EGCG 100                | 39.0 ± 1.2ab           | 70.1 ± 2.6                  | ND                        |
| Rosi 10                 | 41.0 ± 2.4b            | 60.2 ± 3.7                  | ND                        |

| **Rat feeding study**   |                        |                             |                           |
| Control                  | 408.2 ± 5.9a           | 2334.0 ± 67.7               | 22.1 ± 1.3b               |
| EGCG, 0.5%              | 415.8 ± 8.1b           | 2219.9 ± 68.3               | 22.8 ± 1.6b               |
| Rosi, 0.0036%           | 641.3 ± 9.2b           | 2292.5 ± 24.7               | 82.9 ± 2.2b               |

1 Values are means ± SEM. For each study, means in a column with superscripts without a common letter differ, P < 0.05.
2 db/db mice were supplemented with placebo or 0.25%, 0.5%, or 1% w/w EGCG, (n = 9), or 0.0072% rosiglitazone (n = 5) for 7 wk.
3 db/db were administered orally by gavage with placebo or 30 or 100 mg · kg⁻¹ · d⁻¹ EGCG (n = 10) or 10 mg · kg⁻¹ · d⁻¹ rosiglitazone (n = 5) for 2 wk.
4 ND = not determined.
5 ZDF rats were fed a control diet or supplemented with 0.5% EGCG or 0.0036% rosiglitazone (n = 10) for 11 wk.

**Figure 3**  Blood glucose concentrations during an oral glucose tolerance test (oGTT) of untreated ZDF rats, ZDF rats supplemented with 0.5% EGCG, and ZDF rats treated with 0.0036% rosiglitazone (5 mg · kg⁻¹ · d⁻¹) for 10 wk (A). Calculated area under the curve (AUC) of the oGTT (B). Plasma insulin concentrations before and 15, 30, and 45 min after glucose administration (C). Values are means ± SEM, n = 10/group. Means without a common letter differ, P < 0.05.
TABLE 3  Gene expression in liver and adipose tissue of db/db mice supplemented with EGCG or rosiglitazone for 7 wk.1,2

<table>
<thead>
<tr>
<th>Tissue/gene</th>
<th>Control</th>
<th>EGCG, 0.25%</th>
<th>EGCG, 0.5%</th>
<th>EGCG, 1%</th>
<th>Rosi, 0.0072%</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GK</td>
<td>100 ± 13</td>
<td>138 ± 10</td>
<td>147 ± 8</td>
<td>220 ± 15*</td>
<td>193 ± 30*</td>
<td>0.001</td>
</tr>
<tr>
<td>PEPCK</td>
<td>100 ± 11</td>
<td>86 ± 7</td>
<td>61 ± 5</td>
<td>76 ± 10</td>
<td>34 ± 9</td>
<td>0.001</td>
</tr>
<tr>
<td>ACO-1</td>
<td>100 ± 4</td>
<td>100 ± 7</td>
<td>93 ± 1</td>
<td>180 ± 16*</td>
<td>141 ± 18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CPT-1</td>
<td>100 ± 13</td>
<td>149 ± 15</td>
<td>156 ± 6</td>
<td>275 ± 14*</td>
<td>170 ± 21</td>
<td>0.001</td>
</tr>
<tr>
<td>Adipose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEPCK</td>
<td>100 ± 11</td>
<td>55 ± 8*</td>
<td>60 ± 7</td>
<td>56 ± 7*</td>
<td>73 ± 14</td>
<td>0.039</td>
</tr>
<tr>
<td>ACO-1</td>
<td>100 ± 6</td>
<td>63 ± 6</td>
<td>94 ± 17</td>
<td>154 ± 16*</td>
<td>223 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CPT-1</td>
<td>100 ± 6</td>
<td>109 ± 9</td>
<td>113 ± 17</td>
<td>171 ± 16</td>
<td>830 ± 83*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. *Different from control, P < 0.05.
2 Relative mRNA levels of glucokinase (GK), phosphoenolpyruvate carboxykinase (PEPCK), acyl-CoA oxidase-1 (ACO-1), and carnitine palmitoyl transferase-1 (CPT-1) of control db/db mice, and db/db mice supplemented with 0.25%, 0.5%, or 1% w:w EGCG (n = 9/group), and db/db mice treated with 0.0072% rosiglitazone (n = 5) for 7 wk.

and G6Pase in a time- and dose-dependent manner. Expression of PEPCK was reduced by 11.2 or 32.2% after 4 h and 46.6 or 69.2% after 24 h at 50 or 100 μmol/L EGCG, respectively (Table 4). Expression of G6Pase was decreased by 16.1 or 58.1% after 4 h and 47.2 or 87.4% after 24 h at 50 or 100 μmol/L EGCG, respectively (Table 4).

DNA microarray analysis revealed that EGCG significantly modulates expression of genes involved in lipid and glucose metabolism. Overall lipid metabolism was downregulated, as seen by reduced expression of genes involved in fatty acid synthesis (fatty acid synthase and stearoyl-CoA desaturase 1 and 2), oxidation (short-chain acyl-CoA dehydrogenase) and activation (acyl-CoA synthetase 2), as well as triacylglycerol synthesis (glycerol-3-phosphate acyltransferase) and cholesterol synthesis (3-hydroxy-3-methylglutaryl-CoA synthase 1, mevalonate kinase). Genes involved in gluconeogenesis (glucose-6-phosphatase, fructose-1,6-bisphosphatase 1) were downregulated, whereas genes involved in glycolysis (phosphofructokinase) and glucose transport (glucose transporter 1) were increased (Table 5).

Discussion

This study demonstrates that dietary supplementation of EGCG markedly ameliorates T2DM in preclinical models. Thus, to our knowledge, we provide the first evidence that EGCG consumed in the diet exerts potent antidiabetic activity in vivo in a dose-dependent manner.

Our findings clearly show that EGCG enhances oral glucose tolerance in severely diabetic db/db mice and in moderately diabetic ZDF rats. In vivo and in vitro findings suggest that the reduction of EGP and increase in glucose-induced insulin secretion contribute to the antidiabetic effects of EGCG. EGP is the main determinant of fasting glucose levels. EGCG supplementation causes a pronounced decrease of glucose levels in food-deprived animals, which exceeds the decrease of glucose levels in animals not deprived of food. Additionally, the results of a modified insulin tolerance test performed in db/db mice, which were deprived of food just prior to the test, point in the same direction. No effect of EGCG supplementation during the insulin-mediated decrease in blood glucose was observed. Instead, we observed a significant difference in blood glucose starting 3 h after the injection of rapid-acting insulin. The difference was caused by absence of the rebound in blood glucose in EGCG supplemented db/db mice. This again suggests that EGP is decreased by EGCG. Further euglycemic-hyperinsulminemic clamp studies for the determination of EGP, gluconeogenesis, and glycogenolysis will provide a better mechanistic understanding.

Furthermore, our results imply that EGCG supplementation influences the expression of genes involved in glucose and lipid metabolism in the liver as well as in H4IIE rat hepatoma cells. The same pattern of downregulation of gluconeogenic enzymes and upregulation of glycolytic enzymes was found in vitro and in vivo, suggesting that reduced EGP at least partially contributed to the enhanced glucose tolerance of mice supplemented with EGCG. The observed decrease in PEPCK mRNA expression as a key gluconeogenic enzyme is in line with other published in vitro evidence (23).

To our knowledge, we report for the first time that EGCG supplementation causes a dose-dependent increase in GK mRNA expression in livers of db/db mice. In the liver, an increase in GK activity leads to enhanced glycolysis and hepatic glucose uptake (34). However, in a diet-induced obesity model, EGCG supplementation led to reduced expression of GK mRNA in the liver, suggesting that the upregulation of GK observed in our study may be a secondary effect (17).

The increase in insulin concentrations in fed db/db mice supplemented with EGCG could either be caused by direct stimulation of insulin secretion in response to feeding or by a protective effect of EGCG on the pancreas. The results of our study are in line with recently published data, which suggest that EGCG preserves and protects the pancreas by its strong protective effect of EGCG on the pancreas. The results of our study are similar to recent findings and provide an explanation for the antidiabetic activity of EGCG in vivo.

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TABLE 4  Gene expression in H4IIE rat hepatoma cells after treatment with EGCG for 4 or 24 h.1,2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Time</th>
<th>Vehicle (DMSO)</th>
<th>Dex + cAMP</th>
<th>Dex + cAMP + EGCG, 50 μmol/L</th>
<th>Dex + cAMP + EGCG, 100 μmol/L</th>
<th>Dex + cAMP + Ins, 10 nmol/L</th>
<th>% of Dex + cAMP</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEPCK</td>
<td>4</td>
<td>7.6 ± 0.2*</td>
<td>100.0 ± 3.8</td>
<td>88.8 ± 6.6</td>
<td>67.8 ± 4.9*</td>
<td>39.0 ± 2.3*</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>9.2 ± 2.0*</td>
<td>100.0 ± 12.4</td>
<td>63.4 ± 12.4</td>
<td>30.8 ± 3.5*</td>
<td>9.5 ± 6.2*</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>G6Pase</td>
<td>4</td>
<td>41.9 ± 11.6*</td>
<td>100.0 ± 16.2</td>
<td>83.9 ± 14.6</td>
<td>41.9 ± 11.1*</td>
<td>19.1 ± 1.8*</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>30.2 ± 8.1*</td>
<td>100.0 ± 15.0</td>
<td>52.8 ± 10.3</td>
<td>12.6 ± 3.5*</td>
<td>2.5 ± 0.2*</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 3 independent experiments. *Different from Dex + cAMP, P < 0.05.
2 Relative mRNA levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) in H4IIE rat hepatoma cells after treatment for 4 and 24 h with vehicle (0.5% DMSO), 0.5 μmol/L dexamethasone (Dex) + 0.1 nmol/L cAMP (control), 50 and 100 μmol/L EGCG, and 10 nmol/L insulin.
antioxidative capacity (27,28). This would ultimately lead to enhanced pancreatic function and improved insulin secretion in response to feeding. Our study in ZDF rats showed that EGCG enhances glucose-stimulated insulin secretion. This is especially important in preclinical models such as db/db mice and ZDF rats that are characterized by a progressive decline of pancreatic function due to subsequent beta-cell failure and loss of beta-cell mass.

Interestingly, in 2 models of diet-induced obesity, characterized by moderately increased insulin concentrations in fed animals, EGCG supplementation resulted in decreased insulin levels. There are 2 possible explanations for this finding. First, EGCG supplementation could increase insulin concentrations in fed animals only if glucose levels reach pathophysiologically high concentrations, which is not the case in the utilized diet-induced obesity models. Second, the prevention of diet-induced obesity due to EGCG supplementation could reduce peripheral insulin resistance, thereby reducing levels of circulating insulin.

Our results indicate that there is an increase of in vivo fatty acid oxidation due to EGCG supplementation by the upregulation of CPT-1 and ACO-1 mRNA levels in liver and adipose tissue of db/db mice. Furthermore, DNA microarray analysis of H4IIE rat hepatoma cells after treatment with 50 μmol/L EGCG for 24 h revealed the following gene expression changes:

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank no.</th>
<th>Percentage of control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA synthesis</td>
<td>M76767</td>
<td>63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stearyl-CoA desaturase 1</td>
<td>AF509569</td>
<td>72</td>
<td>0.012</td>
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<tr>
<td>Stearyl-CoA desaturase 2</td>
<td>AB032243</td>
<td>68</td>
<td>0.010</td>
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<tr>
<td>Short-chain acyl-CoA dehydrogenase</td>
<td>U64451</td>
<td>52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acyl-CoA-synthetase 2</td>
<td>D90109</td>
<td>60</td>
<td>0.008</td>
</tr>
<tr>
<td>Glycerol-3-phosphate acyltransferase</td>
<td>AF21348</td>
<td>76</td>
<td>0.034</td>
</tr>
<tr>
<td>3-hydroxy-3-methylglutaryl-CoA synthase 1</td>
<td>NM_012768</td>
<td>80</td>
<td>0.043</td>
</tr>
<tr>
<td>Mevalonate kinase</td>
<td>M29472</td>
<td>79</td>
<td>0.042</td>
</tr>
<tr>
<td>Glucose-6-phosphatase, catalytic</td>
<td>U07993</td>
<td>21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphatase 1</td>
<td>J04112</td>
<td>74</td>
<td>0.007</td>
</tr>
<tr>
<td>Phosphofructokinase, liver</td>
<td>NM_013190</td>
<td>128</td>
<td>0.005</td>
</tr>
<tr>
<td>Glucose transporter 1</td>
<td>M13979</td>
<td>157</td>
<td>0.019</td>
</tr>
</tbody>
</table>

1 Values are means, n = 3 independent experiments.
2 Selected genes involved in fatty acid (FA) synthesis, FA oxidation, FA activation, triacylglycerol (TG) synthesis, cholesterol (Chol) synthesis, gluconeogenesis and glycolysis are compared with 0.5% DMSO (percentage of control).

In conclusion, this study demonstrates that among various green tea catechins, EGCG possesses pronounced anti diabetic efficacy in preclinical models of T2DM. The effects of EGCG are at least partially mediated through reduced hepatic glucose production and enhanced pancreatic function. Our data suggest that supplementation with EGCG could potentially improve glucose tolerance in humans with T2DM.

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