Dietary Supplementation with a Low Dose of (−)-Epigallocatechin-3-Gallate Reduces Pro-Inflammatory Responses in Peripheral Leukocytes of Non-Obese Type 2 Diabetic GK Rats

Yumiko UCHIYAMA 1, Takuji SUZUKI 2, Kazuki MOCHIZUKI 1,3 and Toshinao GODA 1, ∗

1 Laboratory of Nutritional Physiology, The University of Shizuoka Graduate School of Nutritional and Environmental Sciences, Shizuoka 422–8526, Japan
2 Department of Information, Environmental and Food Sciences, University of Yamagata, Yamagata 990–8560, Japan
3 Faculty of Life and Environmental Sciences, University of Yamanashi, Yamanashi 400–8510, Japan

(Received June 28, 2013)

Summary (−)-Epigallocatechin-3-gallate (EGCG), which is largely found in green tea, is known to eliminate reactive oxygen species and associated inflammatory responses in vitro and in cells. However, the in vivo mechanisms underlying the effects of EGCG on the amelioration of metabolic disorders are not fully understood. In this study, we examined whether dietary supplementation with EGCG reduces inflammatory responses in peripheral leukocytes of a non-obese type 2 diabetes animal model, Goto-Kakizaki (GK) rats. GK rats at 9 wk of age were fed a control high-fat diet (46 energy % from lard and corn oil) or a high-fat diet containing 0.1%, 0.2%, or 0.5% EGCG (w/w) for 25 wk. The oxidative stress markers 8-hydroxydeoxyguanosine (OHdG) and total malondialdehyde (MDA) were reduced by supplementation with EGCG at 0.1%, but not at 0.2% or more. Significant reductions in the mRNA levels of genes related to inflammatory responses (TNF-α, IFN-γ, IL-1β, IL-6, IL-18, MCP-1, CD11b, and S100a6), 8-OHdG, and total MDA were induced in peripheral leukocytes of GK rats by EGCG supplementation at 0.1%, but not at 0.2% or more, compared with rats fed the control diet. The present results suggest that supplementation with a low dose of EGCG reduces oxidative stress and the expressions of genes involved in inflammation in peripheral leukocytes of GK rats.

Key Words EGCG, peripheral leukocyte, pro-inflammatory cytokine, chemokine, oxidative stress

Hyperglycemia in type 2 diabetes induces many complications, such as nephropathy, decreased pancreatic insulin secretion, insulin resistance, retinopathy, and cardiovascular diseases (CVD) (1). The major causes of these complications induced by hyperglycemia are oxidative stress and activation of leukocytes such as monocytes/macrophages, and the responses are collectively referred to as inflammation (1). Recent studies have shown that hyperglycemia directly induces inflammation by enhancing pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and IL-18, which are mainly expressed by leukocytes, including macrophages, monocytes, and neutrophils, and many peripheral tissues (2–4). These cytokines are known to activate neutrophils/macrophages/monocytes and induce their infiltration into tissues (2). In addition, several studies have shown that the cytokines enhanced by hyperglycemia can directly induce inflammation without infiltration of leukocytes into tissues, such as the findings that several cytokines induce apoptosis in many tissues by activating the caspase cascade (5). Indeed, it has been established that apoptosis of islet β-cells is induced by cytokines, such as IL-1β and interferon (IFN)-γ, resulting in an increased risk of type 2 diabetes development by reducing the capacity for insulin secretion (6, 7). In addition, these cytokines induce macrophage infiltration into the vascular endothelium and increase the risk of developing CVD (8, 9). Furthermore, one of the major causes of insulin resistance is thought to be the production of cytokines, in particular IL-1β, IL-6, and TNF-α (2). These observations indicate that pro-inflammatory cytokine production in leukocytes under diabetic conditions induces the progression of type 2 diabetes and related complications.

Several recent studies have demonstrated that the pro-inflammatory cytokine expression in leukocytes is induced by reactive oxygen species (ROS) and the associated oxidative stress (10, 11). It has been reported that hyperglycemia induces ROS production mainly by enhancing activation of the mitochondrial respiratory chain (12). It appears likely that ROS production under diabetic conditions enhances pro-inflammatory cytokine expression, and that elimination of ROS may reduce the expression of pro-inflammatory cytokines in leukocytes.

Many studies have suggested that antioxidant biofactors would eliminate ROS and reduce oxidative stress. Among the antioxidant biofactors, a number of episodes...
mental and epidemiological studies have reported that (−)-epigallocatechin gallate (EGCG), which is a major catechin in green tea, can reduce the risk of chronic diseases such as CVD (13). It was reported that consumption of green tea extracts or EGCG has beneficial effects on blood glucose control in obese and type 2 diabetic humans (14), mice (15), and rats (16). It has already been shown that EGCG eliminates ROS in vitro (17, 18) and reduces 8-hydroxydeoxyguanosine (OHdG), which is a product of DNA oxidation by ROS (19). It appears likely that intake of EGCG or green tea reduces pro-inflammatory cytokine expression in leukocytes, and that the reduction leads to decreased development of type 2 diabetes and related complications.

Although green tea and EGCG presumably have several beneficial effects on health, higher doses of green tea or EGCG may cause unknown adverse effects (20). It has been reported that higher consumption of green tea or catechins, in particular by supplementation, can exert liver injury (21). Animal studies revealed that higher intake of EGCG causes oxidative DNA damage in the hamster pancreas and liver (22). Consequently, the issue of whether optimal concentrations of EGCG can reduce oxidative stress-associated inflammation and the development of type 2 diabetes and its complications needs to be investigated.

In this study, we examined whether optimal doses of EGCG can reduce pro-inflammatory cytokine expression in peripheral leukocytes of a non-obese diabetes model, Goto-Kakizaki (GK) rats, which show moderate post-prandial hyperglycemia with lower insulin secretion, similar to Asian people including Japanese.

**MATERIALS AND METHODS**

**Animals.** Male 4-wk-old GK rats and age-matched male Wistar rats were purchased from Japan SLC, Inc. (Hamamatsu, Japan). All animals were housed at 22±3°C under a 12-h/12-h light/dark cycle, and allowed free access to a standard laboratory chow (CE-2; CLEA Japan, Inc., Tokyo, Japan). At 9 wk of age, GK rats were assigned to one of four groups (n=8 per group) with similar fasting serum glucose levels (309±3 mg/dL, mean±SE) and body weights (387±8 g), and fed either a control diet or a diet containing 0.1%, 0.2%, or 0.5% (w/w) EGCG preparation for 25 wk (Table 1). The GK rats were fed the control diet as a reference for 25 wk. The rats were euthanized by decapitation. The experimental procedures used in the present study conformed to the Guidelines of the Animal Usage Committee of the University of Shizuoka.

**RNA extraction from peripheral leukocytes.** Blood samples were obtained from the tail vein, and immediately mixed with PAXgene fixation solution for RNA, which is able to immediately fix leukocytes without changing the mRNA levels (Qiagen/BD, Tokyo, Japan). The blood samples in fixation solution were incubated at room temperature for 24 h in accordance with the manufacturer’s instructions. Total RNA was extracted from the blood samples isolated from peripheral leukocytes (250 ng) using a RNeasy Kit (Qiagen) in accordance with the manufacturer’s instructions. Total RNA was stored at −80°C for subsequent quantitative RT-PCR analyses.

**Quantitative RT-PCR.** We used a PAXgene Blood system (Qiagen) that enables leukocyte mRNA expression to be measured, because this system immediately stabilizes blood RNA as previously described (23). Total RNA samples isolated from peripheral leukocytes were converted to cDNA by reverse transcription using SuperScript™ III RT (Invitrogen) in accordance with the

---

**Table 1. Compositions of the experimental diets.**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet</th>
<th>0.1%</th>
<th>0.2%</th>
<th>0.5%</th>
<th>kcal/kg</th>
<th>energy %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg</td>
<td>g/kg</td>
<td>g/kg</td>
<td>g/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>800</td>
<td>16.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>1,000</td>
<td>20.6</td>
</tr>
<tr>
<td>α-Cornstarch</td>
<td>199.5</td>
<td>199.5</td>
<td>199.5</td>
<td>199.5</td>
<td>798</td>
<td>16.5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>450</td>
<td>9.3</td>
</tr>
<tr>
<td>Lard</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>1,800</td>
<td>37.1</td>
</tr>
<tr>
<td>AIN-93 vitamin mix¹</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIN-93G mineral mix¹</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-Cystine</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>49</td>
<td>48</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGCG²</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>4,848</td>
<td>100</td>
</tr>
</tbody>
</table>

¹ AIN-93 vitamin mix and AIN-93G mineral mix (J Nutr 1993; 123: 1939–1951) were prepared by Oriental Yeast, Co., Ltd. (Tokyo Japan).

² Sunphenon EGCg (Taiyo Kagaku, Mie, Japan) is extracted from green tea leaves, and contains >90% EGCG and <5% other catechins (<4% epicatechin gallate).
The PCR primer sequences are listed in Table 2. The amplification protocol consists of denaturation of DNA at 95°C for 10 s, activation of Taq DNA polymerase and denaturation of the library system associated with the LightCycler Instrument (Roche Molecular Biochemicals, Tokyo, Japan). Real-time RT-PCR in this study.

### Table 2. Sequences of oligonucleotide primers used for RT-PCR in this study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>5'-AGCATGATCGAGATGTGGA-3' 5'-AAGGAAAGGCTGCTGAC-3'</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>5'-GGTTTGACGCTGCTCATG-3' 5'-TTTTCAAATGATGTGCTCC-3'</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5'-AAAATGCTCTGCTGCTGCA-3' 5'-CAGGGAATTGTGCTGCTG-3'</td>
</tr>
<tr>
<td>IL-6</td>
<td>5'-CACGACACTTTCTGAGACTCCA-3' 5'-ACTGACCAGGTGCTTCTGGT-3'</td>
</tr>
<tr>
<td>MCP-1</td>
<td>5'-CAGAAACCAGCCAACTCCTCA-3' 5'-TCGAGGGGCTTAACACTC-3'</td>
</tr>
<tr>
<td>CD11a</td>
<td>5'-TCAGTTCCACAGTTCCAGC-3' 5'-AGCTCCATACACACATCTG-3'</td>
</tr>
<tr>
<td>CD11b</td>
<td>5'-ACTCTGATGCTCTGCTGTCG-3' 5'-GCTGAGACAGGGTCTTTCTGC-3'</td>
</tr>
<tr>
<td>CD11c</td>
<td>5'-AACGCCAAGTGGTCCTTCG-3' 5'-CAGGAGCTGTGGCGGTCTG-3'</td>
</tr>
<tr>
<td>CD18</td>
<td>5'-GGGCTGCTGATGAACTCACAT-3' 5'-AATGACGTGTGGCGGTCTG-3'</td>
</tr>
<tr>
<td>S100a4</td>
<td>5'-AGCTACTGACACAGCTGCTG-3' 5'-CTGGAATGCAGCTTCGCTC-3'</td>
</tr>
<tr>
<td>S100a6</td>
<td>5'-AAGGGGCGTACAGCCTGAC-3' 5'-TTTCACGCACTTGGCAGTTA-3'</td>
</tr>
<tr>
<td>YY1</td>
<td>5'-CAGACACTTTGGCAGACTTCA-3' 5'-TCAGTTCCCAGTCAAGTCCAGC-3'</td>
</tr>
</tbody>
</table>

The mRNA levels of selected genes, PCR amplifications were performed using a LightCycler Instrument (Roche Molecular Biochemicals, Tokyo, Japan). Real-time RT-PCR amplifications were carried out in a total volume of 10 μl containing 400 nM each of gene-specific primers (Rikaken, Aichi, Japan), cDNA, and LightCycler 480 Probes Master (Roche Molecular Biochemicals). The real-time PCR amplifications of cDNA from peripheral leukocytes were performed using a universal probe library system associated with the LightCycler Instrument. The amplification conditions were as follows: activation of Taq DNA polymerase and denaturation of DNA at 95°C for 5 min; and 45 cycles of a three-step PCR program (denaturation of DNA at 95°C for 10 s, annealing at 60°C for 25 s, and extension at 72°C for 1 s). The PCR primer sequences are listed in Table 2. The cycle threshold (CT) values for each gene and an internal control gene for a transcriptional repressor protein (YY1) detected by real-time RT-PCR were converted into signal intensities by the delta-delta method (24), which calculated the difference of one CT value as a two-fold difference between each signal for the gene and the signal for the gene for normalization (YY1). The formula is [2^(-ΔΔCT) of gene / 2^(-ΔΔCT) of test gene].

Other assays. Blood was collected from the tail tips of rats after an 8-h fast before and at 24 wk after starting the experimental diets. The serum levels of glucose, 8-OHdG, and total malondialdehyde (MDA) were measured using commercial kits, namely the Glucose CII-test Wako Kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan), High sensitive 8-OHdG ELISA Kit (Japan Institute for the Control of Aging, Shizuoka, Japan), and Colorimetric TBARS Microplate Assay Kit (Funakoshi Co. Ltd., Japan), respectively. Measurements of glycoalbumin levels and activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed by SRL Inc. (Tokyo, Japan).

### Statistical analyses.

The results were expressed as means±SE. Statistical analyses were performed using one-way analysis of variance (ANOVA) and a post-hoc Tukey’s multiple-range test. Differences were considered to be significant for values of p<0.05. All statistical analyses were performed using Excel Statistics software version 2007 for Windows (Social Survey Research Information Co. Ltd., Tokyo, Japan).

### RESULTS

#### Effects of feeding rats diets supplemented with EGCG on food intake and weight gain

In this study, GK rats were fed a control diet or a diet containing 0.1%, 0.2%, or 0.5% EGCG for 25 wk. The mean food intake and weight gain during the experimental period did not differ among the GK rat groups, while the food intake of GK rats fed the control diet or the diets supplemented with EGCG was significantly greater than that of Wistar rats fed the control diet (Table 3).

#### Effects of feeding rats diets supplemented with EGCG on blood glucose parameters

The glycoalbumin concentration was higher in GK control rats than in Wistar rats. The fasting serum glucose concentration was significantly higher (p<0.05) in GK rats fed the control diet than in Wistar rats, while the serum glucose concentration tended to be lower in GK rats fed the diet containing 0.1% EGCG than in GK rats fed the control diet. The fasting serum insulin concentration tended to be lower in GK rats fed the control diet than in Wistar rats, but the serum insulin concentration was significantly higher (p<0.05) in GK rats fed the diet containing 0.1% EGCG than in GK rats fed the control diet. The triglyceride concentration and activities of ALT and AST in the blood were significantly lower in all GK rat groups than in Wistar rats, and did not differ among the GK rat groups (Table 3).

#### Effects of feeding rats diets supplemented with EGCG on oxidative stress markers in blood

The 8-OHdG and total MDA concentrations were sig-
nificantly higher (p<0.05) in GK rats fed the control diet than in Wistar rats. Supplementation of the diet with 0.1% EGCG significantly reduced the serum concentrations of 8-OHdG and total MDA by 38% and 53%, respectively, compared with control GK rats. The serum concentrations of 8-OHdG and total MDA did not differ among GK rats fed the diets containing 0.2% and 0.5% EGCG, and those fed the control diet (Fig. 1A).

Effects of EGCG on the mRNA levels of pro-inflammatory cytokines and S100 proteins in the peripheral leukocytes of rats

Supplementation of the diet with EGCG at 0.1%, but not at 0.2% or more, significantly reduced the mRNA levels of IFN-γ, IL-1β, II-6, II-18, and monocyte chemoattractant protein (MCP)-1 by 82%, 62%, 77%, 76%, and 53%, respectively, in peripheral leukocytes, compared with GK rats fed the control diet. The mRNA levels of CD11b were significantly lower in GK rats fed the diet containing EGCG at 0.1%, but not at 0.2% or more, than in GK rats fed the control diet. Supplementation of the diet with EGCG at 0.1%, but not at 0.2% or more, significantly reduced the mRNA levels of S100a6 by 69% in the peripheral leukocytes, compared with GK rats fed the control diet (Fig. 1B).

**DISCUSSION**

It has been reported that higher postprandial hyperglycemia is positively associated with the production of oxidative stress and the subsequent development of type 2 diabetes and related complications. In particular, Japanese people tend to show increased postprandial hyperglycemia (25), and this postprandial hyperglycemia would induce associated oxidative stress. Our previous studies in humans and animals revealed that reduction of postprandial hyperglycemia by an α-glucosidase inhibitor, miglitol, reduces the expressions of pro-inflammatory cytokine genes, such as IL-1β and TNF-α, in peripheral leukocytes of a diabetic animal model and type 2 diabetic patients (26, 27). Since it has been reported that hyperglycemia induces the production of ROS and associated oxidative stress, and that ROS induces the expressions of inflammatory cytokine genes such as IL-1β and TNF-α (28–30), it is very likely that elimination of ROS and oxidative stress will reduce the expressions of inflammatory cytokine genes such as IL-1β and TNF-α in peripheral leukocytes.

In this study, we examined the effects of an antioxidant EGCG, a catechin that reportedly eliminates ROS in vitro (17, 18) and reduces oxidative stress markers such as 8-OHdG and total MDA in humans and animals. Interestingly, we found that supplementation with 0.1% EGCG reduced the gene expressions of inflammatory cytokines (TNF-α, IFN-γ, IL-1β, II-6, and II-18), integrins (CD11b), S100 proteins (S100a6), and MCP-1 in peripheral leukocytes of GK rats. The indicated cytokines are known to activate neutrophils/macrophages/monocytes and induce their infiltration into many tissues, including adipose tissues (2). It has been reported that S100 proteins, such as S100a8 and S100a9, can activate macrophages/monocytes and induce the infiltration of macrophages into many tissues (2). MCP-1 is known to induce the migration of leukocytes, including monocytes, and higher expression of MCP-1 was reported to be found in human atherosclerotic lesions (31). Thus, supplementation with 0.1% EGCG reduced the inflammatory responses in peripheral leukocytes, and this repression may reduce the risk of development of type 2 diabetes and related complications, in particular arteriosclerosis-related diseases such as CVD.

It should be noted that we have demonstrated in this study that EGCG supplementation at 0.1% reduces the expression of pro-inflammatory cytokine genes
Effects of EGCG on Cytokine Expressions

Effects of EGCG on Cytokine Expressions

in peripheral leukocytes without a significant change in blood glucose concentration. These results suggest that a supplementation with optimal concentrations of EGCG suppresses the hyperglycemia-mediated ROS production and subsequent inflammation in various tissues including peripheral leukocytes, which are likely to be associated with progression of diabetic complications. Indeed, a recent study in humans suggested that green tea may contribute to reductions in the risk of type 2 diabetes and CVD (14). In a cross-sectional study, an inverse association between green tea consumption and coronary atherosclerosis was observed in 512 Japanese...
subjects undergoing coronary angiography (32). In a cohort study on green tea consumption, it was found that the risk of significant stenosis was lower by 50% in subjects consuming 2–3 cups per day and by 60% for those consuming ≥4 cups per day, compared with those consuming ≤1 cup per day in a subgroup of 262 men without dietary or drug treatments for type 2 diabetes (32). In addition, a retrospective cohort study among 17,413 Japanese adult subjects found a 33% reduction in the risk of developing type 2 diabetes in subjects consuming ≥6 cups of green tea per day, compared with those consuming <1 cup per week (33). These lines of evidence indicate that green tea consumption has a strong inverse association with the development and progression of oxidative stress-related diseases such as type 2 diabetes and arteriosclerosis-related diseases. It should be examined in further studies whether the optimal concentration of EGCG for a longer period reduces the risk of diabetic complications including CVD, and improves glycemic control as well.

Although we did not determine EGCG concentrations in the plasma of GK rats fed diets supplemented with EGCG at concentration of 0.1%, 0.2% and 0.5% in this study, a previous study showed that the plasma concentration of EGCG in C57BL/10-mdx mice fed a diet containing 0.1% EGCG for 5 wk was 29.7 ± 3.2 ng/mL (34). Therefore, the GK rats fed a diet supplemented with 0.1% EGCG may have similar plasma EGCG concentration as in the previous study in C57BL/10-mdx mice. Further studies should examine the plasma concentrations of EGCG attained by the administration of diets supplemented with 0.1%, 0.2% and 0.5% EGCG. We have also demonstrated in this study that plasma concentrations of 8-OHdG, a product of DNA oxidation induced by ROS, and MDA, a marker of increased lipid peroxidation, were both reduced by 0.1% EGCG supplementation in GK rats, suggesting that supplementation with EGCG at 0.1% EGCG reduces oxidative stress and may have eliminated ROS produced by hyperglycemia in GK rats. This result is in accordance with previous human studies which showed that consumption of green tea catechins reduced plasma concentration of 8-OHdG and TNF-α (19).

In this study, EGCG supplementation at 0.2% or more in the diet was unable to reduce the expression of genes related to inflammatory responses in peripheral leukocytes. Although green tea has several beneficial effects on health, the effects of green tea and its constituents may be beneficial up to a certain dose, and higher doses may cause unknown adverse effects. It has been reported that EGCG and green tea extracts are cytotoxic, and that higher consumption of green tea can exert acute cytotoxicity toward cells in the liver, a major metabolic organ in the body (21). Animal studies found that higher intake of green tea caused oxidative DNA damage in the hamster pancreas and liver (22). Therefore, higher intake of green tea may be detrimental for diabetic animals to control hyperglycemia. In addition, our results indicate that the optimal concentrations of antioxidants, including EGCG, can be determined by measuring the expressions of genes related to inflammation in peripheral leukocytes. Thus, the technique of measuring the expressions of genes related to inflammation in peripheral leukocytes would be useful for estimations of the efficacy and safety of food factors including EGCG and drugs in humans as well as in animals.

To determine whether our results are applicable to humans, the human equivalent of the doses used in this study needs to be mentioned. Based on the average daily food intake of the GK rat (17 g), a diet containing 0.1% EGCG provided rats with a daily intake of 17 mg EGCG, or 50 mg/kg for a rat of 340 g (average BW in the current study). When this dose is converted from rats consuming 68 kJ/d to humans consuming 1,120 kJ/d using an isocaloric calculation (35, 36), it is roughly equivalent to 4 mg/kg/d in humans, or 280 mg EGCG per day consumed by a 70-kg person. Likewise, a high dose of 0.5% of EGCG for rats in this study is equivalent to human daily consumption of 1,400 mg. A cup of green tea contains 150–180 mg EGCG, and commercially available EGCG supplements contain up to 350 mg EGCG/tablet. Thus, the low dose (0.1%) used for GK rats in the current study is achievable in humans who consume EGCG as a supplement or by drinking 2–3 cups of green tea. Further studies should examine the reason(s) why higher concentrations (0.2% or 0.5%) of EGCG failed to suppress the mRNA levels of inflammatory cytokines.

In conclusion, we have demonstrated in this study that dietary supplementation with EGCG at a relatively low level (0.1%), but not at higher levels, suppresses hyperglycemia-induced inflammatory responses by reducing the expression of genes related to inflammation in peripheral leukocytes.

Acknowledgments

This work was supported by the global COE program, the Center of Excellence for Evolutionary Human Health Sciences, from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES


