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Original Article

Supplementation with lower doses of EGCg reduces liver injury markers of type 2 diabetic rats

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ABSTRACT — (-)-Epigallocatechin-3-gallate (EGCg), a major catechin in green tea, eliminates reactive oxygen species and development of lifestyle-related diseases. However, excessive EGCg intake could induce adverse effects, particularly liver injury. We examined whether optimal dietary doses of EGCg reduces the risk of liver injuries in non-obese type 2 diabetic Goto-Kakizaki (GK) rats by examining gene expression of the proinflammatory cytokines interleukin (IL)-1β, IL-18 and tumor necrosis factor-α (TNF-α) and fibrosis-related matrix metalloproteinases (MMPs) in the liver. GK rats at 9 weeks of age were fed a control high-fat diet or a high-fat diet containing 0.1%, 0.2% or 0.5% EGCg (w/w) for 25 weeks. Expression of mRNA and proteins related to inflammation were determined by qRT-PCR and western blot analysis, respectively. IL-1β and IL-18 mRNA in the liver were reduced by EGCg supplementation at concentrations of 0.1% and 0.1%-0.2%, respectively, but not at concentrations of 0.2% and 0.5% (IL-1β) or 0.5% (IL-18) EGCg. TNF-α mRNA in the liver was reduced by supplementation with EGCg at concentrations of 0.1%-0.5%. Expression of MMP2 in the liver was reduced by EGCg supplementation at a concentration of 0.2%, but not 0.1% or 0.5%. Importantly, IL-18 protein levels in the liver and serum were reduced by 0.1% EGCg, but not by 0.5%. EGCg supplementation at concentrations from 0.1% to 0.5% did not induce increases in the expression of liver injury marker genes, while low doses (0.1%–0.2%) of EGCg in GK rats reduced expression of injury-associated genes in the liver.

Key words: (-)-Epigallocatechin-3-gallate, Liver injury, Proinflammatory cytokine, Matrix metalloproteinase, Oxidative stress, Type 2 diabetes

INTRODUCTION

Recent studies have demonstrated that intake of antioxidants is effective in reducing the development of lifestyle-related diseases including obesity and type 2 diabetes. Among antioxidants, a number of experimental and epidemiological studies have reported that (-)-epigallocatechin gallate (EGCg), which is a major catechin in green tea, can reduce the development of lifestyle-related diseases including obesity, type 2 diabetes, and cardiovascular diseases (Babu and Liu, 2008). A randomized clinical trial (RCT) containing 67 participants aged 48 years in the United States demonstrated that consumption of a drink containing 625 mg catechins once a day for 12 weeks enhanced reduction of abdominal fat, free fatty acid, and triglycerides in the blood by exercise com-

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pared with a drink containing 39 mg caffeine (Maki *et al*., 2009). In addition, a meta-analysis of 22 RCTs demonstrated that intake of the green tea catechin reduced fasting blood glucose, without altering fasting insulin concentration, hemoglobin A1c and homeostasis model assessment of insulin resistance (Zheng *et al*., 2013). In animal models, consumption of green tea extracts or EGCg improved blood glucose control in obese and type 2 diabetic mice and rats (Igarashi *et al*., 2007a; Kao *et al*., 2000; Wolfram *et al*., 2006). In addition, EGCg reduced diet-inducible obesity in mice (Byun *et al*., 2014). The inhibition of the development of these diseases or their amelioration by EGCg is presumably at least partially caused by the effects of antioxidants. It has already been shown that EGCg eliminates reactive oxidant species (ROS) *in vitro* (Hu and Kitts, 2001; Nakagawa, 2002; Raza and John, 2007; Rice-Evans *et al*., 1996). The intake of green tea by humans reduces lipid peroxides and 8-hydroxydeoxyguanosine, which is a product of DNA oxidization by ROS (Oyama, 2010). In addition, it has been reported that green tea extract improves lipid abnormalities by activating β-oxidation in the skeletal muscle of mice (Murase *et al*., 2005). Because oxidative stress induces inactivation of β-oxidation, it is very likely that the amelioration of obesity, the related lipid abnormalities and impaired glucose tolerance by EGCg or green tea could be caused by the actions of antioxidants. Our recent studies have demonstrated that a low dose of EGCg (0.1%) in the diet reduced expression of proinflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-18, in peripheral leukocytes and adipose tissues in non-obese, type 2 diabetic Goto-Kakizaki (GK) rats (Uchiyama *et al*., 2013a, 2013b). It has been reported that hyperglycemia induces the expression of these proinflammatory cytokines (Lu *et al*., 2007; Shoelson *et al*., 2006; Wen *et al*., 2006), resulting in development of type 2 diabetes and related complications including cardiovascular disease. These results indicate that optimal intake of EGCg could suppress the development of metabolic diseases and improve established disease by reducing oxidative stress in the body.

However, it has also been reported that higher consumption of supplements containing catechins can cause liver injuries (Mazzanti *et al*., 2009). Indeed, consumption of flavonoids including baicalin and catechins at 250 mg or 500 mg twice daily in four women with arthrosis deformans for 1-3 months induced elevation of circulating liver injury markers including alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin, and these markers were improved at 3-12 weeks after stopping the consumption of supplements (Chalasani *et al*., 2012). It has also been reported that among 57 cases of liver injury caused by herbal supplements from 2004 to 2010 in the United States, that 50.5% of cases included catechins in the supplements (Navarro *et al*., 2013). Animal studies demonstrated that increased intake of EGCg caused oxidative DNA damage in the hamster pancreas and liver (Takabayashi *et al*., 2004). Therefore, the optimal dose of supplements containing catechins still needs to be determined.

Recent studies have demonstrated that proinflammatory cytokines can contribute to the development of liver injury. Treatment of rats with carbon tetrachloride, which causes acute liver injury with fibrosis, induced IL-1β and TNF-α mRNA expression in the liver and protein expression in the blood (Chen *et al*., 2012). In addition, a high-fat diet induced IL-18 in the liver of rats (Wang *et al*., 2008). It has been reported that primary hepatocytes with steatosis secrete IL-18 and TNF-α (Pan *et al*., 2015). Genetic deficiency of the TNF receptor or IL-18 reduced fat accumulation and liver damage in mice (Lana *et al*., 2016). In addition, matrix metalloproteinases (MMPs) have protective effects in liver injury. It was reported that bone marrow stem cells injected into rats with liver fibrosis induced by carbon tetrachloride treatment differentiate into hepatic parenchymal cells, express MMP9 and reduce liver fibrosis (Sakaida *et al*., 2004; Terai *et al*., 2003). In contrast, higher hepatic gene expression and serum levels of MMP2 in patients with nonalcoholic fatty liver disease were positively correlated with steatohepatitis (Toyoda *et al*., 2013). It has been reported that a high-fat diet induces expression of IL-18, TNF-α, and MMPs in the liver (de Meijer *et al*., 2012; Mardare *et al*., 2016). These results suggest that proinflammatory cytokines and MMPs are expressed by damaged hepatocytes, including in the moderate stages of damage, and that expression of IL-18, TNF-α, and MMPs in the liver could be useful for the assessment of liver injuries when testing the effectiveness and safety of new drugs.

In this study, we assessed the optimal doses of EGCg by investigating liver injury markers, including proinflammatory cytokines and MMPs, in the liver of a nonsobese type 2 diabetes model, the Goto-Kakizaki (GK) rat. Notably, this model displays a marked postprandial hyperglycemia with lower insulin secretion, similar to Asian people with type 2 diabetes, including the Japanese.

MATERIALS AND METHODS

Animals

Male 4-week-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 with a 12-hr/12-hr light/dark cycle, and allowed free access to a standard laboratory chow (CE-2; CLEA Inc., Tokyo, Japan). At 9 weeks of age, GK rats were assigned to one of four groups (n = 8 per group) and fed either a control diet or a diet containing 0.1%, 0.2%, or 0.5% (w/w) EGCg preparation (Table 1) for 25 weeks. The EGCg preparation, Sunphenon EGCg (Taiyo Kagaku, Mie, Japan), consisted of > 90% EGCg and < 5% other catechins. The experimental procedures used in the present study conformed to the Guidelines of the Animal Usage Committee of the University of Shizuoka. Detailed protocols for maintaining the GK rats in this study were reported in Uchiyama *et al*. (2013a, 2013b).

RNA extraction and qRT-PCR

The liver total RNA samples were stored at −80°C for subsequent qRT-PCR analyses. Total RNA was extracted by the acidified guanidinium thiocyanate method, as described by Chomczynski and Sacchi (2006). The total RNA samples were converted to cDNA by reverse transcription using SuperScript™ III RT kit (Invitrogen, Waltham, MA, USA) in accordance with the manufacturer's instructions. To quantitatively estimate the mRNA levels of selected genes, PCR amplifications were performed in a LightCycler Instrument (Roche Applied Science, Penzberg, Germany). qRT-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 Applied Science). 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 (TBP) detected by qRT-PCR were converted into signal intensities by the delta-delta CT method (Livak and Schmittgen, 2001), which calculates the difference of one CT value as a two-fold difference. The formula is $[2^{(\text{CT of TBP} - \text{CT of test gene})}]$.

Western blot analysis

Total tissue proteins in the liver were extracted in RIPA buffer [20 mM Tris/HCl, pH 8.0, 5 mM EDTA, 150 mM NaCl, 1% NP-40, 0.1% sodium dodecyl sulfate (SDS) and proteinase inhibitor tablets (cOmplete mini, EDTA-free; Roche Applied Science, Basel, Switzerland)]. Lysates were centrifuged at 17,800 × *g* for 30 min at 4°C. The soluble supernatants were normalized for total protein concentration using the Lowry method, and stored at −80°C. Tissue extracts or serum were separated by 10% SDS-PAGE and transferred to Immobilon membrane (Merck Millipore, Billerica, MA, USA) at 80 V for 90 min in Tris/glycine/methanol transfer buffer. The membranes were blocked for 30 min in 3% skim milk in PBS with 0.05% Tween 20, pH 7.4 (PBS-Tween) at room temperature. They were then incubated in 3% skim milk PBS-Tween with primary antibody at 4°C for > 7 hr, using anti-IL-18 antibody (Medical and Biological Laboratories, Nagoya, Japan) and anti-General transcription factor IIB (TFIIB) antibody (Santa Cruz Biotechnology, Dallas, TX, USA). After washing in PBS-Tween, the membranes were incubated with anti-rabbit IgG conjugated to biotin (GE Healthcare, Little Chalfont, United Kingdom) in 3% skim milk PBS-Tween. After washing in PBS-Tween and incubating with streptavidin-conjugated horseradish peroxidase (Cell Signaling Technology, Danvers, MA, USA), signals were detected by chemiluminescence (Western Lightning ECL Pro; PerkinElmer, Waltham, MA, USA),

Table 1. Composition of the experimental diets.

	Control diet	EGCg diet		
		0.1%	0.2%	0.5%
Ingredient	g/kg	g/kg	g/kg	g/kg
Casein	200	200	200	200
Sucrose	250	250	250	250
α-Corn starch	199.5	199.5	199.5	199.5
Corn oil	50	50	50	50
Lard	200	200	200	200
AIN93 vitamin mix	35	35	35	35
AIN93G mineral mix	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5
L-Cystine	3	3	3	3
Cellulose	50	49	48	45
EGCg	0	1	2	5
Total	1000	1000	1000	1000

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

Gene	Sequence
Interkeukin-1β (IL-1β)	5`-AAATGCCTCGTGCTGTCTGA-3` 5`-CAGGGATTTTGTCGTTGCTTG-3`
Interkeukin-18 (IL-18)	5`-CAGACCACTTTGGCAGACTTCA-3` 5`-ACACAGGCGGGTTTCTTTTGT-3`
Interkeukin-12a (IL-12a)	5`-CACTCCCAAAACCTGCTGAG-3` 5`-TCTCTTCAGAAGTGCAAGGGTA-3`
Interkeukin-12b (IL-12b)	5`-AGACCCTGCCCATTGAACT-3` 5`-TGATGAAGAAGCTGGTGCTG-3`
Tumor necrosis factor (TNF)-α	5-AGCATGATCCGAGATGTGGAA-3′ 5-AATGAGAAGAGGCTGAGGCACA-3′
Matrix metalloproteinase 2 (MMP2)	5`-TGATAACCTGGATGCAGTCG-3` 5`-AGCACCCTTGAAGAATAGCTG-3`
Matrix metalloproteinase 9 (MMP9)	5`-GTTTCTGCCCCAGTGAGAAT-3` 5`-CATCCGAGCGACCTTTAGTG-3`
TATA-binding protein (TBP)	5’-CCCACAACTCTTCCATTCTCA-3’ 5’-TTTGGAGCTGTGGTACAATCC-3’

according to the manufacturer’s instructions. Immunoblotted membranes were scanned by a luminal image analyzer (LAS3000, Fujifilm, Tokyo, Japan).

Statistical analyses

The results are expressed as means ± standard error of the mean (SEM). Statistical analyses were performed using Student’s *t*-test or one-way analysis of variance (ANOVA) and a *post hoc* Tukey multiple-range test. Differences between groups were considered statistically 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

Basic parameters for food intake, weight gain, and blood parameters in rats fed a control diet and diets including 0.1%, 0,2% and 0.5% EGCg for 25 weeks are described in our previous reports (Uchiyama *et al*., 2013a, 2013b). The mean food intake and weight gain were not differed among groups of EGCg-treated GK rats. The fasting concentration of blood glucose (356 ± 37 mg/dL in GK-control, 296 ± 19 mg/dL in 0.1% EGCg GK-group, 328 ± 33 mg/dL in 0.2% EGCg GK-group, and 344 ± 24 mg/dL in 0.5% EGCg GK-group) and insulin (3.2 ± 0.2 ng/mL in GK-control, 5.1 ± 0.4 ng/mL in 0.1% EGCg GK-group, 3.6 ± 0.3 ng/mL in 0.2% EGCg GK-group, and 3.1 ± 0.3 ng/mL in 0.5% EGCg GK-group). The ALT, and aspartate aminotransferase (AST) were also not different among groups of EGCg-treated GK rats.

mRNA levels of IL-1β and IL-18, but not TNF-α, were higher in GK control rats without EGCg treatment than in Wistar rats. mRNA of IL-1β and IL-18 were reduced by supplementation with EGCg at concentrations of 0.1% and 0.1%-0.2%, respectively. The expression of these cytokines was not reduced at concentrations of 0.2% and 0.5% (IL-1β), or 0.5% (IL-18) EGCg. In contrast, the mRNA level of TNF-α was reduced by EGCg supplementation at concentrations of 0.1%-0.5%. mRNA levels of IL-12a and IL-12b, the two components of the IL-12 heterodimer which induces inflammation and differentiation of Th1 cells from Th0 cells, were not altered by the EGCg treatments. Gene expression of MMP2 was higher in GK control rats than in Wistar rats and the levels in GK rats was reduced by supplementation with EGCg at a concentration of 0.2% EGCg but not of 0.1% and 0.5%. mRNA levels of MMP9 were not altered by EGCg treatment (Fig. 1).

Notably, protein levels of IL-18 in both the liver and serum were higher in untreated GK rats than in control Wistar rats. IL-18 protein levels in the liver and serum were also reduced by 0.1% EGCg but not by 0.5% EGCg (Figs. 2-3).

DISCUSSION

Our previous studies in humans and animal models demonstrated that reducing postprandial hyperglycemia with the α-glucosidase inhibitor miglitol suppressed the expression of proinflammatory cytokine genes, including IL-1β and TNF-α, in peripheral leukocytes of an ani-

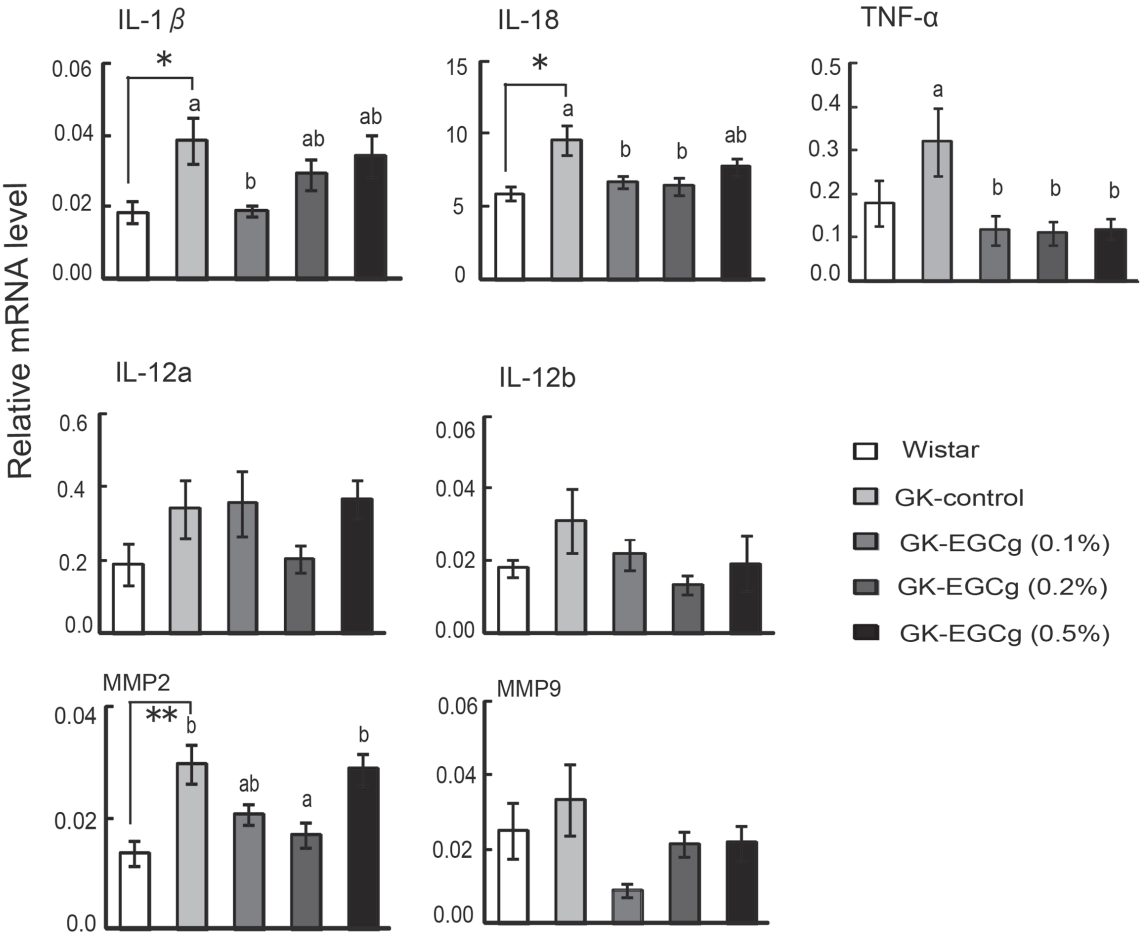

Fig. 1. Effects of dietary supplementation with EGCg on expression of genes related to inflammation (IL-1β, IL-18, TNF-α, IL-12a, IL-12b, MMP2, and MMP9) in the livers of GK rats fed a control high-fat diet or a high-fat diet containing 0.1%, 0.2%, or 0.5% EGCg. Asterisks indicate significant differences between Wistar rats and GK control-rats by two-sided unpaired Student's *t*-test (* $P < 0.05$, ** $P < 0.01$). Conditions not sharing a common superscript letter ([a], [b]) differ significantly among GK groups by Tukey's multiple-range test ($P < 0.05$).

mal model with diabetes and patients with type 2 diabetes (Osonoi *et al*., 2010; Tanaka *et al*., 2009). The expression of proinflammatory molecules induced by hyperglycemia is thought to be caused by ROS, because hyperglycemia induces ROS production (Ceriello, 2003; Zhang *et al*., 2001) and ROS induces the expression of inflammatory cytokines such as IL-1β and TNF-α (Dasu *et al*., 2007; Kempf *et al*., 2007; Reape and Groot, 1999). Indeed, our previous studies demonstrated that dietary EGCg at a relatively low level (0.1%), but not at higher levels (0.2%-0.5%), reduced expression of proinflammatory cytokines including IL-1β, TNF-α, and IL-18 in the peripheral leukocytes of GK rats (Osonoi *et al*., 2010; Tanaka *et al*., 2009). IL-1β and TNF-α secreted from activated leukocytes induces atherosclerosis by upregulating production of E-selectin, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1, which are adhesion molecules of the vascular endothelium (Bartzeliotou *et al*., 2007; Ginn-Pease and Whisler, 1998; Haddad, 2002), resulting in enhancement of leukocyte attachment to the vascular endothelium. Combined with these results, our previous studies investigating the effects of EGCg on leukocytes suggest that optimal EGCg intake could reduce atherosclerosis.

In this study, we examined whether optimal dietary doses of EGCg reduces the risk of liver injuries in non-obese type 2 diabetic GK rats. We further demonstrated in the current study that treatment with EGCg reduced gene

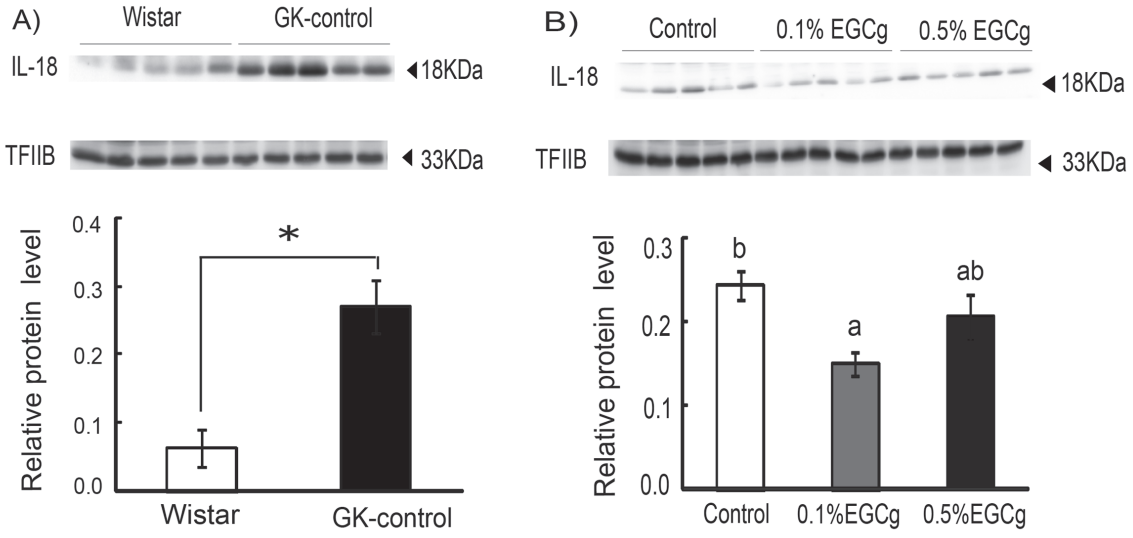

Fig. 2. Effects of dietary supplementation with EGCg on expression of IL-18 protein in the livers of GK rats fed a control diet or a diet containing 0.1%, or 0.5% EGCg. (A) Comparison between Wistar rats and GK control group. (B) Comparison among GK groups. IL-18 protein levels were normalized by TFIIB protein levels. Asterisks indicate significant differences between Wistar rats and GK control-rats by two-sided unpaired Student's *t*-test (* $P < 0.05$). Conditions not sharing a common superscript letter ([a], [b]) differ significantly among GK groups by Tukey's multiple-range test ($P < 0.05$).

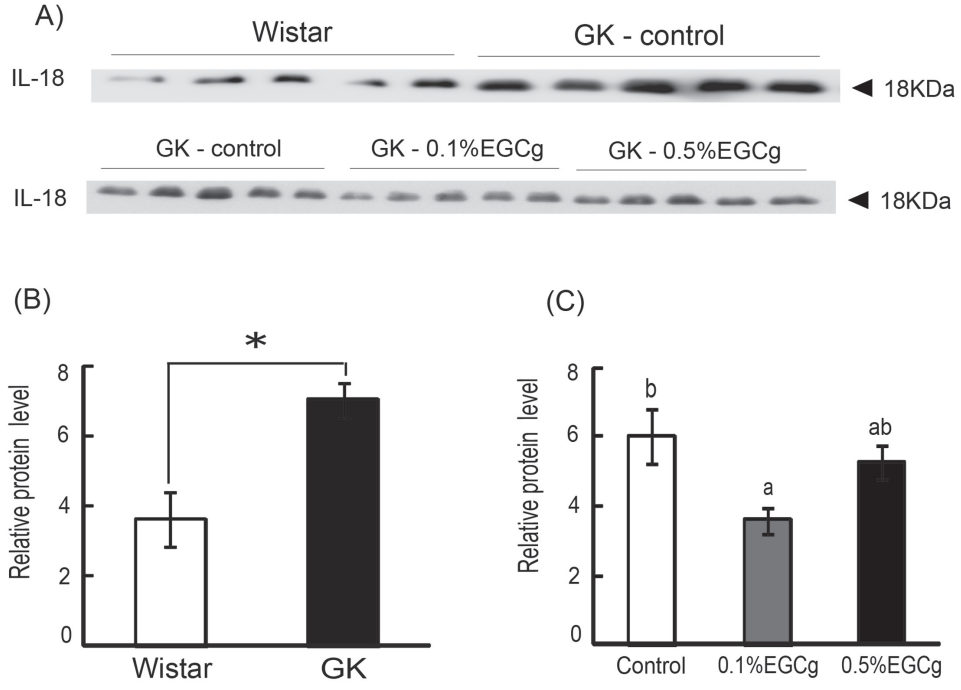

Fig. 3. Effects of dietary supplementation with EGCg on expression of IL-18 protein in serum of GK rats fed a control diet or a diet containing 0.1%, or 0.5% EGCg. (A) Electrophoresis image. (B) Comparison between Wistar rats and GK control group. (C) Comparison among GK groups. Asterisks indicate significant differences between Wistar rats and GK control-rats by two-sided unpaired Student's *t*-test (* $P < 0.05$). Conditions not sharing a common superscript letter ([a], [b]) differ significantly among GK groups by Tukey's multiple-range test ($P < 0.05$).

expression of IL-1β (0.1%), TNF-α (0.1%-0.2%), IL-18, and MMP2 (0.2%), and EGCg-treatment at a concentration of 0.1%, but not 0.5%, reduced IL-18 protein in the liver and serum. In addition, we have shown in a previous report that the oxidative stress markers 8-hydroxydeoxyguanosine (OHdG) and total malondialdehyde (MDA) in serum were reduced by supplementation with EGCg at 0.1%, but not at 0.2% or 0.5%, in the GK rats (Uchiyama *et al*., 2013b). Because the expression of these genes induces liver damage, including fatty liver and fibrosis, optimal EGCg concentration reduces the risk of causing liver damage. In this study, we did not find any difference in general serum liver injury markers including ALT and AST. Thus, the expression of IL-1β, IL-18, and MMP2 could more sensitively detect liver injury. Importantly, IL-18 protein levels in the liver were closely positively correlated with IL-18 protein levels in the serum. Thus, determination of IL-18 protein in the serum may detect liver injury in humans. Indeed, the serum concentration of IL-18 was closely associated with liver fibrosis in patients with chronic hepatitis C (Selim *et al*., 2009). Therefore, the association between serum IL-18 concentration and liver injury in patients with type 2 diabetes or impaired glucose tolerance should be examined. It should also be determined whether EGCg treatment at doses greater than 0.5% and longer treatment at concentrations less than 0.5% alters the expression of genes related to inflammation in the liver and liver injury markers in serum and the liver. In addition, it is still unclear whether protein levels of IL-1β, TNF-α, and MMP2 in the liver were altered by EGCg supplementation in the GK rats. This should be examined in further studies.

Interestingly, expression of TNF-α, but not IL-1β, IL-18, and MMP2, was reduced by 0.5% EGCg. These results indicate that higher concentrations of EGCg may not be effective in reducing liver injury. In this study, we did not find adverse effects with 0.5% EGCg. However, expression of IL-1β, IL-18, and MMP2 was higher or tended to be higher at 0.5% EGCg than at 0.1%-0.2% EGCg. Thus, over 0.5% EGCg may induce proinflammatory cytokine expression and liver injury compared with untreated rats. Therefore, it should be investigated whether greater than 0.5% EGCg in the diet induces proinflammatory cytokine expression in the liver and causes liver injury.

Notably, EGCg supplementation at concentrations of 0.1%, 0.2% and 0.5% in the diet of GK rats for 16 weeks did not alter 8-h-fasted glucose concentration, glucose concentration in the oral glucose tolerance test, glycoalbumin, and HbA1c (Uchiyama *et al*., 2013a, 2013b). These results indicate that EGCg supplementation at 0.1%, 0.2%, and 0.5% for 16 weeks in GK rats did not alter glycemic control. Igarashi *et al*. demonstrated that 0.2% dietary catechins in GK rats for 76 days ameliorated impaired glucose tolerance (Igarashi *et al*., 2007b). The differences between the study by Igarashi *et al*. and our studies were that fasting blood glucose concentration was under 200 mg/dL, and 1-hr glucose concentration was under 500 mg/dL in EGCg untreated rats in the study by Igarashi *et al*., whereas in our study, fasting blood glucose concentration was over 200 mg/dL, and 1-hr glucose concentration was over 500 mg/dL in EGCg untreated rats. Thus, type 2 diabetes was more severe in our studies than in the study by Igarashi *et al*. EGCg may ameliorate dysregulated glycemic control during the stages of impaired glucose tolerance and mild type 2 diabetes. However, our current study in conjunction with our previous studies indicate that optimal concentrations of EGCg supplement reduced oxidative stress and inflammation in the liver, adipose tissues and blood in the GK rats. Reduction of oxidative stress and inflammation in type 2 diabetes could reduce the incidence of diabetic complications, such as nephropathy, retinopathy, neuropathy, cardiovascular diseases, and steatohepatitis. Thus, it should be investigated whether EGCg supplementation at optimal concentrations reduces these complications in animals and humans.

Our previous studies showed that GK rats fed 0.1%, 0.2% and 0.5% EGCg consumed approximately 50, 100 and 250 mg/kg BW, respectively. When those values are converted to a human scale using an isocaloric calculation, they are equivalent to 280 mg (0.1%), 560 mg (0.2%), 1400 mg (0.5%) EGCg per day consumed by a 70-kg person (Uchiyama *et al*., 2013a, 2013b). A cup of green tea contains 150-180 mg EGCg, and commercially available EGCg supplements contain up to 350 mg EGCg/tablet. Thus, regular supplements containing EGCg or 2-3 cups of green tea may reduce liver injuries in humans with type 2 diabetes, but over 1400 mg EGCg would induce adverse effects. Further studies should examine the association between EGCg supplementation and serum IL-18 protein expression or liver injury in humans at various ages and disease stages in multiple geographic regions.

In conclusion, we have demonstrated in this study that dietary supplementation with EGCg at a relatively low level (0.1%-0.2%), but not at higher levels, suppresses the proinflammatory cytokines IL-1β, TNF-α and IL-18, and MMP2 in the liver. Our results suggest that supplementation with 0.1% to 0.5% EGCg does not induce increases in liver injury markers and low doses (0.1%-0.2%) of EGCg in GK rats actually reduces liver injury markers in the liver.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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