Effects of Dietary Catechins on Glucose Tolerance, Blood Pressure and Oxidative Status in Goto-Kakizaki Rats

Kiharu Igarashi1, Keisuke Honma1, Orie Yoshinari2, Fumio Nanjo1 and Yukihiko Hara3

1Department of Bioresource Engineering, Faculty of Agriculture, Yamagata University, 1–23, Wakaba-machi, Tsuruoka, Yamagata 997–8555, Japan
2Course of the Science of Bioresources, The United Graduate School of Agricultural Science, Iwate University, Morioka, Iwate 020–8550, Japan
3Mitsui Norin Co., Ltd., Miyabara, Fujieda, Shizuoka 426–0133, Japan

Summary Effects of green tea catechins comprising EGCg, EGC, ECg, EC, GCg, GC, Cg, and C were determined on blood glucose tolerance and oxidative stress status in type 2 diabetic Goto-Kakizaki (GK) rats. GK rats fed the catechin-containing diet tended to maintain blood glucose and systolic blood pressure at lower levels in the latter stages of the feeding period of 76 d, compared to those not receiving dietary catechins (control group). The blood glucose tolerance test performed on days 48–49 showed that GK rats fed the catechins had lower blood glucose levels than GK rats not fed catechins during the 120 min after glucose loading. In catechin-fed rats, amounts of 8-OH dG and albumin excreted into the urine determined on days 71–72, and kidney ACE activity determined on day 76, were lower than those in control rats. From these results it is concluded that dietary catechins may be effective in delaying the progression of diabetes and the associated oxidative stress.

Key Words catechins, diabetes, Goto-Kakizaki rat, glucose tolerance, oxidative stress

Catechins, which are especially abundant in green tea, have attracted much attention for their many physiological functions such as hypcholesterolemic effects (1), protective effects against liver injury (2) and oxidative stress (3), anti-mutagenic activity (4), anti-tumor activity (5), and improvement of glucose tolerance (6–8). However, long term-feeding effects of tea catechins including EGCg, EGC, ECg, EC, GCg, GC, Cg, and C on glucose tolerance, blood pressure and oxidative status, especially in genetically modified type 2 diabetic GK rats, have not yet been studied. GK rats are known as models of non-insulin dependent diabetes mellitus (Type 2 diabetes) and are particularly relevant to the understanding of human type 2 diabetes, because mild hyperglycemia has been demonstrated as early 2 to 4 wk after birth in these animals. It is also known that until 12 mo of age, GK rats exhibit a moderate and stable fasting hyperglycemia, which does not progress to a ketotic state. Therefore, at the early stage of diabetes, the GK rat does not present the severe complications associated with this condition. It is thus an appropriate model to study events occurring at the onset of diabetes, compared to obese diabetic rats, which present with severe hyperglycemia and hyperlipidemia (9).

In this study, the effects of tea catechins on glucose tolerance and oxidative stress markers such as serum and organ thiobarbituric acid-reactive substances (TBARS), urinary 8-hydroxy deoxyguanosine (8-OH dG) and albumin levels, and angiotensin I converting enzyme (ACE) activity were determined in GK rats. Serum lipid levels and blood pressure levels which are known to be increased in the development of diabetes accompanying nephrosis (10) were also determined.

MATERIALS AND METHODS

Preparation of catechins. Polyphenone E prepared at Mitsui Norin Co., Ltd. was the source of catechins used in this experiment. The purity of catechins was about 89% on a dry weight basis, being composed of 65.6% EGCg, 2.8% EGC, 6.8% ECG, 9.2% EC, 3.8% GCg, 0.2% GC, 0.2% Cg, 0.8% C.

Animal maintenance and measurements of blood glucose, blood pressure levels, and glucose tolerance. Male GK rats (body weight: 130–160 g; 8 wk old) were purchased from Clea Japan, Inc. (Tokyo, Japan). Rats were kept in an air conditioned room at 22±2°C with 40–60% humidity and a 12 h light-dark cycle (light cycle: 0600–1800 h). After acclimatization for 3 d, the rats were divided into 2 groups of 5 each, fed on either a basal diet or a basal diet containing catechins. The composition of the experimental diets is shown in Table 1. The diet and water were given ad libitum during the experimental period of 76 d. The body weight was measured every other day. Blood glucose levels were measured every other week in blood collected from the tail vein after 7 h fasting, using Medesafe GR-102 (Termo Co. Ltd., Tokyo, Japan). Blood pressure was measured every other week by the tail cuff method with a pro-
grammed electro sphygmomanometer (BP-98; Softron Co., Tokyo, Japan) after placing the rat in a heat box set at 38°C for 10 min after 6 h fasting.

The glucose tolerance test was carried out twice: firstly in rats fasting for 12 h on day 48–49 of the feeding period for the first glucose-loading test and secondly in rats fasting for 12 h on day 73–74 for the second glucose-loading test. A 20% d-glucose solution in distilled water was administered orally, generating a dose of 2 g/kg. The glucose tolerance in normal male Wistar strain rats at 10 wk age (body weight 233 ± 4 g) that were fed on a commercial diet (F2, Funahashi, Japan) was also measured in the same manner for comparison with that of GK rats.

Rats were cared for at all times according to the institutional guidelines of Yamagata University.

**Measurement of insulin, adiponectin levels and ACE activity.** Blood was collected by cardiac puncture from rats anesthetized with Nembutal (Dainippon Pharmaceutical Co., Osaka, Japan) after 10 h fasting following the feeding period of the experimental diet (76 d). Following blood collection, liver and kidney were harvested and stored at −80°C until required for analysis. Serum was prepared by centrifuging blood at 3,000 × g for 10 min. Serum levels of insulin and adiponectin were assayed using respective ELISA kits (Levis rat insulin kit, Shibaygi, Gunma, Japan; mouse/rat adiponectin ELISA kit, Otsuka Pharmaceutical, Tokyo, Japan).

To prepare enzyme solutions for measurement of kidney ACE activity, 0.7 g of frozen kidney tissue was homogenized in 10 volumes of 0.2 M borate buffer (pH 8.0) in a glass homogenizer, followed by centrifugation at 10,000 × g for 20 min at 4°C. ACE activity in the supernatant was measured according to the method described by Saito et al., using hippuryl-L-histidyl-L-leucine as substrate (11). One unit of ACE activity is defined as nmol hippuric acid formed per min at 37°C per g of liver under assay conditions (12).

**Measurements of TBARS, 8-OH dG and albumin.** For blood TBARS measurement, 0.1 mL of blood collected as described above, was mixed with 1.9 mL of physiological saline, followed by centrifugation at 3,000 × g for 10 min. TBARS content in the supernatant was measured according to the method of Yagi (13) and was expressed as nmol malondialdehyde per mL of blood. The concentrations of liver and kidney TBARS were measured by the method of Uchiyama and Miura using the homogenate, which was prepared by homogenizing 0.5 g of the frozen liver or kidney with 9 volumes of cold 1.15% KCl solution (14).

To determine urinary 8-OH dG, the urine collected for 48 h during days 71–72 was centrifuged at 3,000 × g for 20 min and the precipitate was discarded. The content of 8-OH dG in the supernatant was measured using an 8-OH dG ELISA kit (Japan Institute for the Control of Aging, Fukuroi, Japan) (15, 16). Creatinine content in the supernatant was measured using a commercial kit (Creatinine-test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan). The content of 8-OH dG in the urine was expressed relative to the content of creatinine (ng/mg of creatinine).

Albumin content in the urinary supernatant was measured using a commercial kit. (Albumin B-test Wako, Wako Pure Chemical Industries).

**Lipid analyses.** Total and HDL-cholesterol, and triacylglycerol in serum were measured enzymically using commercial kits (cholesterol E-test, HDL-cholesterol test, and triglyceride E-test, respectively, Wako Pure Chemical Industries).

**Statistical analyses.** Values are given as means ± SE. Inter-group differences were determined using a Student’s t-test. A p value <0.05 was taken to indicate significant differences between means.

**Table 1. Composition of experimental diet (%).**

| Constituent | Control diet | Catechins diet |
|-------------|--------------|----------------|
| Casein      | 15.0         | 15.0           |
| α-Cornstarch: sucrose = 2:1 | 69.5 | 69.3 |
| Corn oil    | 5.0          | 5.0            |
| Cellulose   | 5.0          | 5.0            |
| Vitamin mixture<sup>a</sup> | 1.0 | 1.0 |
| Mineral mixture<sup>b</sup> | 3.5 | 3.5 |
| NaCl        | 1.0          | 1.0            |
| Polyphenone E | 0.2 |    |

<sup>a</sup> Control diet, basal diet group without catechins; Catechin diet, basal diet group with catechins. Catechins were composed of 65.6% EGCg, 2.8% EGC, 6.8% EGc, 9.2% EC, 3.8% GCg, 0.2% GC, 0.2% Cg, and 0.8% C.  
<sup>b</sup> AIN-93G-MX and AIN-93-VX which contained 25 g bitartrate per 100 g were obtained from Oriental Yeast Co., Ltd.

**Fig. 1.** Changes over time in (A) blood glucose and (B) systolic blood pressure levels of GK rats fed dietary catechins. ● control group without dietary catechins; ■ group fed dietary catechins. *Significantly different from the control group, p < 0.05. Blood glucose levels at day 0 were not determined.
RESULTS

Blood glucose and systolic blood pressure levels over time

Blood glucose and systolic blood pressure levels over the 10-wk feeding period are shown in Fig. 1A and B respectively. Although blood glucose and systolic blood pressure levels in the first half of the feeding period showed irregular changes, those in the latter half (after 6 wk) showed lower values, or a tendency to be lowered in the catechin group compared to the control group. Blood glucose levels in the catechin-fed group in the latter half period were significantly lower than that of the control group at 6 and 9 wk, and systolic blood pressure of the catechin-fed group showed significantly lower values at week 9 compared to that of the control group.

Glucose tolerance

The effects of catechins on blood glucose tolerance determined after feeding the experimental diet for 7 (day 48–49) and 10 wk (day 73–74) are shown in Fig. 2A and B respectively. At week 7, increases in blood glucose levels continuing for 60 min after glucose loading were significantly less at 15, 30 and 60 min in the catechin-fed group than in the control group. Furthermore, the glucose levels from 60–180 min was also significantly less in the catechin-fed group than in the control group. Increase in blood glucose level at 15, 30 and 60 min in both groups was clearly higher than that of the control group. Increase in blood glucose level at 15, 30 and 60 min in the catechin-fed group showed significantly lower values, or a tendency to be lowered compared to the control group. Further half period were significantly lower than that of the control group at 6 and 9 wk.

Blood glucose and systolic blood pressure levels over time

(A) Glucose loading was carried out (15 wk old) in GK rats fed a diet with or without catechins for 48–49 d. Normal Wistar strain rats (10 wk old, male) fed a commercial diet were also measured for glucose tolerance in the same way as the GK rats. (B) Glucose loading was carried out (18 wk old) in GK rats fed a diet with or without catechins for 73–74 d. Blood glucose levels in the catechin-fed group in the latter part were significantly lower than that of the control group, although blood glucose levels on day 76 and glucose tolerance on day 73–74 did not differ between the control and catechin-fed groups.

Table 2. Effects of dietary catechins on oxidative status, insulin and adiponectin levels, and angiotensin I converting enzyme activity, and serum lipid levels of GK rats.

| Dietary group | Control | Catechins |
|---------------|---------|-----------|
| Initial body weight (g) | 147±3 | 144±5 |
| Food intake (g/76 d) | 1.17±0.22 | 1.17±0.12 |
| Body weight gain | 159±18 | 162±7 |
| Liver weight (% of body weight) | 2.2±0.1 | 2.9±0.1 |
| Kidney weight (% of body weight) | 0.79±0.02 | 0.81±0.01 |
| TBARS | | |
| in blood (nmol/mL of blood) | 7.52±0.51 | 5.81±0.59 |
| in liver (nmol/g of liver) | 132±7 | 128±7 |
| in kidney (nmol/g of kidney) | 144±2 | 136±11 |
| 8-OH dG | | |
| in urine (×10^-8 mg/mg creatinin) | 28.0±1.7 | 15.3±3.4** |
| Albumin | | |
| in urine (mg/48 h) | 71.6±5.4 | 49.7±7.4* |
| Insulin | | |
| in serum (pg/mL of serum) | 562±157 | 968±156 |
| Adiponectin | | |
| in serum (ng/mL of serum) | 3.28±0.10 | 3.29±0.05 |
| ACE activity | | |
| in kidney (U/g of kidney) | 2.07±0.14 | 1.43±0.05* |
| in blood (nmol/mL of serum) | 22.7±2.4 | 28.6±2.1 |
| Serum lipids | | |
| Total cholesterol (mg/dL) | 109±5.4 | 95.0±3.5 |
| HDL-cholesterol (mg/dL) | 72.2±3.5 | 74.0±3.1 |
| Atherogenic index | 0.52±0.04 | 0.29±0.06* |
| Triacylglycerol (mg/dL) | 51.2±5.3 | 53.2±4.1 |

GK rats 8 wk of age were given the diet with 2% catechins for 76 d. The amounts of 8-OH dG and creatinine were measured using the urine collected during from 71st to 72nd days, and the other components and enzyme activity were measured by using the samples collected at 76th day (the day was serum collected and organs detached).

*Significantly different from the corresponding control group at p<0.05. **0.05<p<0.1 vs. control group.

Table 2. Effects of dietary catechins on oxidative status, insulin and adiponectin levels, and angiotensin I converting enzyme activity, and serum lipid levels of GK rats.

GK rats 8 wk of age were given the diet with 2% catechins for 76 d. The amounts of 8-OH dG and creatinine were measured using the urine collected during from 71st to 72nd days, and the other components and enzyme activity were measured by using the samples collected at 76th day (the day was serum collected and organs detached).

*Significantly different from the corresponding control group at p<0.05. **0.05<p<0.1 vs. control group.

Oxidative status

TBARS levels in the blood, liver and kidneys were measured using the urine collected during from 71st to 72nd days, and the other components and enzyme activity were measured by using the samples collected at 76th day (the day was serum collected and organs detached).

*Significantly different from the corresponding control group at p<0.05. **0.05<p<0.1 vs. control group.

Insulin, adipokine and atherogenic index

Insulin and adiponectin levels in the serum did not differ between the control and catechin-fed groups, in
spite of the fact that the oxidative stress marker 8-OH dG and albumin were affected by the feeding of catechins.

Although serum triacylglycerol levels were not affected by feeding catechins, the atherogenic index showed significantly lower values in the catechin-fed group, compared with that of the control group.

**DISCUSSION**

The lower levels of both blood glucose and systolic blood pressure in the catechin-fed rats, especially in the latter half of the feeding period, may suggest that catechins have the ability to protect against the progress of diabetes and improve glucose tolerance; furthermore, an improvement of glucose tolerance may be associated with an improvement in blood pressure (17). However, the possibility is not excluded that antidiabetic and blood pressure-suppressing effects were demonstrated by the respective independent action route (6, 18). Lower levels of kidney ACE activity in the catechin-fed rats may partly support the view that ACE inhibitory activity of catechins might be responsible for the lower systolic blood pressure levels seen compared with that of the control group (19).

Feeding of catechins to GK rats for 7 wk improved glucose tolerance; however the level of glucose tolerance at week 10 (day 73–74) of catechin-feeding did not differ significantly between the control and catechin-fed groups (Fig. 2A and B). Since it is known that insulin resistance in the GK rat is improved gradually with age (16), a reason for the lack of differences in glucose tolerance between control and catechin-fed groups at week 10 might be due to aging, since the rats were 18 wk old at this point. In addition it may be that diabetes had progressed to a more severe insulin-resistant stage that can not be mitigated by the feeding of catechins, by this time.

The lower levels of urinary 8-OH dG in the catechin-fed group may indicate that catechins act to suppress the progress of oxidative DNA degradation which occurs in increased oxidative stress. A suppressive effect of catechin on urinary secretion of 8-OH dG in GK rats has not been reported in spite of the fact that catechin has as antidiabetic effect in mice (8). Insulin and adiponectin levels in the serum did not differ between the control and catechin-fed groups; this was in spite of the fact that the oxidative stress marker 8-OH dG and urinary albumin content were increased in the control group. This may indicate that catechins cannot restore insulin or adiponectin levels that might have been affected by the more severe progress of diabetes (20). It is reported that ingestion of catechins over a long period rather increases serum insulin levels in patients with diabetes (21). It is also reported that the feeding of EGCg to ob/ob mice over 7 wk lowers plasma cholesterol level but increases plasma insulin level, and that the protective effect of EGCg against pancreatin oxidation enhances pancreatin function to increase insulin secretion in response to feeding (8). On the other hand, it is known that dietary catechins suppress the increase in serum insulin levels induced by feeding α-cornstarch; furthermore, catechins increase insulin activity, resulting in a decrease in serum insulin levels (22, 23). These different reports may indicate that the effects of dietary catechins on serum insulin levels in diabetes may differ depending on the degree of severity of the condition. Therefore, it may be impossible from this study alone to clarify the reasons for the lack of difference in insulin levels between the control and catechin-fed groups at the final stage of the study (day 76). However, improvement of glucose tolerance at 7 wk (day 48–49) and lower urinary albumin excretion level at day 71–72 in catechin-fed GK rats, compared to those of control Gk rats, may support the possibility that dietary catechins suppress the progress of diabetes, in spite of further investigation being necessary to confirm an anti-diabetic effect of catechins.

Although, it is considered that inhibition by EGCg of intestinal glucose uptake by sodium-dependent glucose transport, SGLT1 (7), and a direct effect of EGCg on hepatic glucose metabolism (8) are concerned in the improvement of diabetes by EGCg, those effects of EGCg were not investigated in this study. The lower atherogenic index in catechin-fed rats may indicate that catechins are able to suppress the atherogenesis that might be caused by diabetes.

Although glucose tolerance was not significantly improved by the dietary catechins in the second glucose-loading test (day 73–74), the other biomarkers such as TBARS, and urinary 8-OH dG and albumin levels which were determined on day 76 and 71–72, respectively, showed lower values in the catechin-fed rats. These results may indicate that these biomarkers (except for glucose tolerance) are more influenced by longer feeding with catechins.

This study may indicate that dietary catechins improve glucose tolerance and oxidative status in GK rats as diabetes progresses. It would be of interest to determine which components in the catechin mixture are the most effective in suppressing the progress of oxidation in diabetes. However, it should be stressed that a larger contribution of EGCg might be expected because of its relative abundance and because its protective effect against paraquat-induced oxidative stress is the greatest among the group comprising EGCg, ECG, EGC and EC (24).

**REFERENCES**

1) Fukuyo M, Hara Y, Muramatsu K. 1986. Effects of tea leaf catechin, (−)-epigallocatechin gallate on plasma cholesterol level in rats. Nippon Eijo Shokuryou Gakkaishi 39: 495–500 (in Japanese).

2) Chen JH, Tiptoe GL, Liong EC, So HSH, Leung KM, Tom WM, Fung PCW, Nanji AA. 2004. Green tea polyphenols prevent toxin-induced hepatotoxicity in mice by down-regulating inducible nitric oxide-derived proxioxidant. Ann J Clin Nutr 80: 742–751.

3) Suzuki O, Araki Y, Igarashi K, Yoshiki Y, Okubo K. 1997. Protective effects of epigallocatechin gallate on paraquat-induced oxidative stress in rats. Food Sci Technol Int Tokyo 3: 150–153.
4) Kada T, Kaneko K, Masuzaki S, Matsuzaki T, Hara Y. 1985. Detection and identification of natural bio-antimutagens, a case of the green tea factor. Nutr Res 150: 127–135.

5) Hara Y, Matsuzaki S, Nakamura K. 1989. Antitumor activity of tea catechins. Nippon Eiyo Shokuryou Gakkai Shi 42: 39–45 (in Japanese).

6) Sakai M, Hara Y. 1995. Functionality of catechin (No. 4). An inhibiting effect of catechin against saccharide decomposing enzymes. Shokuhin Kogyou 38: 77–81 (in Japanese).

7) Wolfram S, Raderstorff D, Preller M, Wang Y, Teixeria SR, Riegger C, Weber P. 2006. Epigallocatechin gallate supplementation alleviates diabetes in rodents. J Nutr 136: 2512–2518.

8) Sabu MC, Smitha K, Ramadasan K. 2002. Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. J Ethnopharmac 83: 109–116.

9) Duhamel J, Koening BD. 1997. Diabetes mellitus and its animal models. Therapie 52: 375–384.

10) Naito Y, Uchiyama K, Akagiri S, Aoi W, Hasegawa G, Nakamura N, Kokura S, Yoshida N, Ichikawa H, Toyokuni S, Yoshikawa T. 2005. Imidapril, an angiotensin-converting enzyme inhibitor, reduces diabetes-induced renal oxidative damage in mice. J Clin Biochem Nutr 37: 29–37.

11) Saito Y, Nakamura K, Kawato A, Imayasu S. 1992. Angiotensin I converting enzyme inhibitors in sake and its by-products. Nippon Nogeikagaku Kaishi 66: 1081–1087 (in Japanese).

12) Liberman J. 1975. Elevation of serum angiotensin-converting enzyme (ACE) level in sarcoidosis. Am J Med 59: 365–372.

13) Yagi K. 1976. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med 15: 212–216.

14) Uchiyama M, Mihara M. 1978. Determination of malondialdehyde precursor in tissue by thiobarbituric acid test. Anal Biochem 86: 271–278.

15) Erhola M, Toyokuni S, Okada K, Tanaka T, Hiai H, Ochi H, Uchida K, Osawa T, Niiminen MM, Alho H, Kellokumpu LP. 1977. Biomarker evidence of DNA oxidation in lung cancer patients: Association of urinary 8-hydroxy-2′-deoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. FFBS Lett 409: 287–291.

16) Toyokuni S, Tanaka T, Hattori Y, Nishiyama Y, Yoshida A, Uchida K, Hiai H, Ochi H, Osaswa T. 1997. Quantitative immunohistochemical determination of 8-hydroxy-2′-deoxyguanosine by a monoclonal antibody N45.1: its application to ferric nitrotriacetate-induced renal carcinogenesis model. Lab Invest 76: 365–374.

17) Shiuchi T, Cui TX, Nakagami H, Takeda-Matsubara Y, Iwai M, Horiuchi M. 2002. ACE inhibitor improves insulin resistance in diabetic mouse via bradykinin and NO. Hypertension 40: 329–334.

18) Hara Y, Tonooka F. 1990. Hypotensive effect of tea catechins on blood pressure of rats. Nippon Eiyo Shokuryou Gakkai Shi 43: 345–348 (in Japanese).

19) Hara Y, Matsuzaki T, Suzuki T. 1987. Angiotensin I converting enzyme inhibiting activity of tea components. Nippon Nogeikagaku Kaishi 61: 803–808 (in Japanese).

20) Koyama M, Wada R, Sakuraba H, Mizutani H, Yagihashi S. 1998. Accelerated loss of islet β-cells in sucrose-fed Goto-Kakizaki rats, a genetic model of non-insulin-dependent diabetes mellitus. Am J Pathol 153: 537–545.

21) Fukuyo Y, Shimbo M, Aoki N, Okubo T, Iso H. 2005. Randomized controlled trial for an effect of green tea consumption on insulin resistance and inflammation markers. J Nutr Sci Vitaminol 51: 335–342.

22) Hara Y, Tonoka F. 1990. A decrease in the angiotensin I converting enzyme (ACE) activity by dietary catechins. Nippon Eiyo Shokuryou Gakkai Shi 43: 345–348 (in Japanese).

23) Anderson R, Dolansky MM. 2002. Tea enhances insulin activity. J Agric Food Chem 50: 7182–7186.

24) Igarashi K, Suzuki O, Hura Y. 1999. Comparison of the protective effects of epigallocatechin gallate, epicatechin gallate and epicatechin on paraquat-induced oxidative stress in rats. Food Sci Technol Res 5: 69–73.